



**Rationale and design of the PRognostic Importance of
MIcrovascular Dysfunction in asymptomatic patients with
Aortic Stenosis (PRIMID-AS)- a multicentre observational
study with blinded Investigations**

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Rationale and design of the PRognostic Importance of MIcrovascular Dysfunction in asymptomatic patients with Aortic Stenosis (PRIMID-AS)- a multicentre observational study with blinded Investigations

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ABSTRACT

Introduction: Aortic stenosis (AS) is the commonest valve disorder in the developed world requiring surgery. Surgery in patients with severe asymptomatic AS remains controversial. Exercise testing can identify asymptomatic patients at increased risk of death and symptom development, but with limited specificity, especially in older adults. Cardiac Magnetic Resonance Imaging (CMR), including Myocardial Perfusion Reserve (MPR) may be a novel imaging biomarker in AS.

Aims: 1. To improve risk stratification in asymptomatic patients with AS, 2. To determine whether MPR is a better predictor of outcome than exercise testing and BNP.

Method/Design: Multi-centre, prospective observational study in the UK, comparing MPR with exercise testing and BNP (with blinded CMR analysis) for predicting outcome. **Population:** 170 asymptomatic patients with moderate-severe AS, who would be considered for Aortic Valve Replacement (AVR). **Primary outcome:** Composite of: typical symptoms necessitating referral for AVR and major adverse cardiovascular events. **Follow-up:** 12-30 months (minimum 12 months). **Primary Hypothesis:** MPR will be a better predictor of outcome than exercise testing and BNP.

Ethics / Dissemination: The study has full ethical approval and is actively recruiting patients. Data collection will be completed in November 2014 and the study results will be submitted for publication within 6 months of completion

ClinicalTrials.gov identifier: NCT01658345.

STRENGTHS

- Study design: blinded imaging with core lab analysis (CMR and exercise testing) to reduce referral bias, inclusion only of patients who would consider surgery, multicentre, data collection/analysis by independent clinical trials unit.
- Comprehensive phenotyping of participants
- Management and oversight: Independent chair and members of steering committee, independent event adjudication
- Harder primary endpoint: asymptomatic patients referred for aortic valve replacement not included
- Registration with NHS Information services to minimise loss to follow-up and acquire long-term data

LIMITATIONS

- Coronary disease not excluded by angiography.
- CMR not widely available in all secondary care settings

INTRODUCTION

Aortic Stenosis (AS) is the commonest valve lesion requiring surgery in the developed world[1]. European registry data indicate that as few as 30% of potentially eligible patients are referred for surgery[2]. As the population in developed countries continues to age, it is predicted that the prevalence of AS will double in the next 20 years[3].

The development of symptoms in AS heralds a malignant phase of the condition and prompt AVR results in a clear reduction in mortality[4]. Surgery in this situation is universally regarded as a class I indication despite the absence of randomised controlled trials[5, 6]. In contrast, management of patients with severe AS in the absence of symptoms remains controversial and continues to provoke debate, with divergent clinical practice[7, 8]. It is generally agreed that the ideal timing of surgical intervention is immediately before a patient develops symptoms. Such a strategy, if successfully implemented, would minimise the risk of sudden death during the long latent (asymptomatic) period and minimise the number of patients sent for surgery who may never have developed symptoms related to AS.

Risk stratification in asymptomatic patients with moderate-severe AS

Many researchers have therefore sought clinical markers in asymptomatic patients with AS, that reliably identify those who will need AVR. The current body of evidence in asymptomatic AS consists mainly of observational studies (Table-1), which have identified a number of risk factors for developing symptoms or death[8,

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3 9]. These include a.) echocardiographic markers: aortic valve calcification, rapid
4 increase in pressure gradient[10], very high aortic valve velocities[11, 12]; b.) an
5 abnormal response or symptoms on exercise testing[13-15]; and c.) abnormal
6 biomarkers, especially Brain Natriuretic Peptides (BNP)[16]. It should be noted
7 however, that echocardiographic measures of AS severity are a poor discriminator
8 between those who go on to develop symptoms and those that do not, compared to
9 other parameters [13, 17].
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22 **Limitations of current research in asymptomatic AS**

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24 The limitations of these research studies have been highlighted in a recent editorial
25 [9]. These include a degree of selection bias due to non-randomisation, unblinded
26 investigations influencing management decisions and patients who subsequently
27 refuse surgery and patients undergoing AVR whilst asymptomatic being included in
28 the primary outcome.
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40 **Exercise testing to risk stratify in AS**

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42 Of the prognostic markers studied to date, most attention has focused on exercise
43 testing[13-15]. A recent meta-analysis of stress testing in 491 asymptomatic patients
44 with severe AS demonstrated that there was no sudden death in those with a normal
45 test, which was also associated with a low risk of subsequent events[18]. However
46 the specificity of a positive test is low for predicting outcome. Das et al showed the
47 positive predictive value of exercise symptoms to be only 57% and even lower in the
48 large and important group of patients >70 years of age[15]. It does seem likely that
49 the mechanisms limiting exercise capacity will be closely related to those that cause
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3 symptoms. However, no trial to date has assessed whether early AVR can improve
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5 outcome in asymptomatic patients with a positive exercise test (or for any other risk
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7 factor). For this reason the ACC/AHA guidelines grade exercise-induced symptoms
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9 as a class IIB indication (can be considered but weight of evidence does not support
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11 intervention) for AVR[19]. Exercise testing has not been widely implemented in
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13 clinical practice.[2]
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20 **Importance of Left Ventricular (LV) remodeling in AS**

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22 The hypertrophic response to pressure overload in severe AS is extremely variable[20,
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24 21]. When left ventricular hypertrophy (LVH) does occur, it is associated with a
25
26 number of detrimental pathophysiological sequelae: reduction in myocardial strain
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28 [22], development of myocardial fibrosis[23] and diastolic dysfunction. These
29
30 changes are also associated with microvascular dysfunction[24]. AS patients with
31
32 high left ventricular mass index (LVMI) are more likely to develop heart failure,
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34 suggesting that adverse ventricular remodeling is promotive of LV systolic
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36 dysfunction[21]. Inappropriately high LVMI has also been identified as an adverse
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38 prognostic marker in patients with severe asymptomatic AS[20].
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45 Following AVR there is regression of LVH which has been associated with
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47 improvement in myocardial strain[22], myocardial perfusion reserve[25] and exercise
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49 capacity[26], but not in all studies[27] However, there are concerns that LVH
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51 regression is incomplete and that myocardial fibrosis may be irreversible leading to
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53 persistent diastolic dysfunction and increased long-term mortality[28].
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Table 1: Prospective studies assessing risk stratification in moderate-severe asymptomatic AS

Author	No.	Severity	CAD	Outcome	Follow-up: months	AVR	Total/ Cardiac Deaths	SCD	Outcome predictor
Rosenhek [10]	128	Severe (>4m/s)	Not excluded	Death, AVR	22±18	59 of 106 (56%);	8 6 cardiac	1 (0.9%)	Calcification, rapid progression
Amato [13]	66	Severe (AVA <1cm ²)	Excluded (angio)	Death, symptoms	15 ±12	?34	4	4 (6.1%)	AVA < 0.7cm ² , positive ETT
Lancelloti [14]	69	Severe (AVA <1cm ²)	Not excluded,	Symptoms, death, AVR	15± 7	12 (17%),	3 cardiac + 1 death post AVR	2 (2.9%) SCD	Exercise mean PG +ve ETT, AVA< 0.75cm ²
Das [15]	125	Moderate to severe(AVA <1.4cm ²),	Not excluded,	Symptoms, death	12	36 (29%) symptoms ? AVR	No deaths	No SCD	Exercise symptoms
Monin [29]	104	Moderate-severe:>3m/s AVA <1.5cm	RWMA excluded	Indication for AVR, death	24	58 AVR	4 deaths (1 post AVR)		Female sex, BNP, peak velocity
Rosenhek [12]	116	Very severe >5m/s	No.	Indication for AVR, death	41 (median)	79 AVR, 10 refused AVR	17 deaths 9 no surgery 8 post AVR	1 SCD	Peak AV >5.5m/s, diabetes, Cholesterol
Kang [11]	197	Very severe >4.5m/s or AVA < 0.75cm ²	History or RWMA	Death	42 AVR 58 medical	148 102 early, 46 (of 95) medical	3 (0 cardiac) early, 28 (12 cardiac) medical	9 (10%) medical 0 early	Peak AV >5m/s
Cioffi [20]	209	Severe (AVA<1cm ² or mean PG>40 mmHg)	History	Death, AVR, MI, HF hospitalisation	22 ± 13	72	20 (16 cardiac)	2 SCD	Inappropriate high LVMI, peak velocity, calcification

Abbreviations: AVA=aortic valve area; PG= pressure gradient; RWMA= regional wall motion abnormalities; AVR= aortic valve replacement; MI= myocardial infarction; HF= heart failure; AV= Aortic valve velocity; BNP= brain natriuretic peptide, angio= coronary angiography; LVMI= left ventricular mass index

(Adapted from [9]-reproduced with permission)

CMR in AS

Focal myocardial fibrosis can be detected non-invasively by late gadolinium enhancement (LGE) in 27-62% of AS patients[30, 31]. The extent of LGE correlates well with, although underestimates the extent of interstitial fibrosis on myocardial biopsy[30, 32] and increases with LVH[30, 31]. LGE in patients with severe AS is associated with limited improvement in symptoms, LV function and higher medium term mortality after AVR.[30, 32]

CMR myocardial tissue tagging can also demonstrate alterations in strain and strain rates, and is considered the gold standard technique for the assessment of function[33]. In AS there is an increase in the normal wringing action (torsion) of the LV, which improves after AVR[34]. The rate of untwisting in AS is reduced which may reduce diastolic filling and myocardial perfusion reserve (MPR) by loss of 'suction' action.

The importance of CMR detected LVH, LGE and MPR in predicting objectively measured maximal aerobic exercise capacity (peak VO_2) in 46 patients with severe isolated AS prior to AVR has been studied[35]. On stepwise regression analysis, MPR was the only independent predictor of sex and age-corrected peak oxygen consumption (peak VO_2), $\beta=0.457$, $p=0.001$. MPR also significantly decreased with increasing New York Heart Association (NYHA) Class ($p=0.001$). Newer CMR methods utilising T1 mapping also offer the possibility of quantifying diffuse myocardial fibrosis not detected by the LGE technique[26]. This has also been shown to be associated with exercise capacity and NYHA class in patients with severe AS prior to AVR[26]. Diffuse fibrosis may be an important determinant of MPR pre-

operatively. However early after AVR there is no significant change in diffuse fibrosis[26, 28] but MPR does increase[25] (reflecting changes in pressure overload and reverse LV remodeling[35]).

MPR is an attractive biomarker in AS since it is dependent on a combination of factors that include: valve severity and measures of LV remodelling/fibrosis and perfusion time. The primary aim of the PRIMID AS study is to assess whether MPR (and other CMR measures) can improve risk stratification in asymptomatic patients with moderate to severe AS, by comparing them to the best studied prognostic indicators: exercise testing and NT-proBNP.

STUDY DESIGN

This is a multi-centre, prospective trial with blinded analysis of CMR data. The trial has been registered on ClinicalTrials.gov (NCT01658345).

Aims of the Study

- To improve risk stratification in asymptomatic patients with AS.
- To determine whether MPR is a better predictor of outcome than exercise testing and NT-proBNP.
- To establish the determinants of MPR in asymptomatic AS.

Two substudies will:

- assess the reproducibility of MPR measurement in AS.

- assess the rate of progression in LV remodelling at 1 year in asymptomatic AS.

Primary Hypothesis

MPR will be a better predictor of adverse outcome than exercise testing in asymptomatic patients with moderate-severe AS.

Inclusion and Exclusion Criteria

These are shown in Table 2.

Table 2: Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Moderate-severe AS (≥ 2 of: AVA $< 1.5\text{cm}^2$, peak pressure gradient $> 36\text{mmHg}$, mean PG $> 25\text{mmHg}$).	History of CABG or recent MI within 6 months.
Asymptomatic.	Previous valve surgery.
Age > 18 years and < 85 years.	Severe valve disease other than AS.
Prepared to undergo AVR if symptoms develop.	Persistent Atrial fibrillation or flutter.
Ability to perform bicycle exercise test.	Severe Asthma.
	History of Heart Failure.
	Severe renal impairment eGFR $< 30\text{ml/min}$.
	Planned AVR.
	EF $< 40\%$.
	Any absolute contraindication to CMR.
	Contraindication to Adenosine.
	Other medical condition that limits life expectancy or precludes AVR
	Pregnancy.

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3 In addition, 20 asymptomatic controls without known cardiac disease will undergo
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5 baseline assessment to allow determination of age and sex-matched normal ranges for
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7 MPR, diffuse myocardial fibrosis and exercise capacity.
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10 11 12 13 **Primary Outcome Measures**

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15 Composite of typical AS symptoms necessitating referral for AVR, cardiovascular
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17 death and Major Adverse Cardiovascular Events (MACE) (hospitalisation with any of:
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19 heart failure, chest pain, syncope, arrhythmia or stroke) at 12 months (time to first
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21 event). Asymptomatic patients having AVR for other reasons (valve progression,
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23 positive exercise test) will be excluded from primary endpoint analysis.
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31 **Secondary Outcome Measures**

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33 1. Composite of AVR (for any reason) and MACE at 12 months (first event).
- 34
35 2. Composite of AVR and MACE during follow-up (first event).
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37 3. Determinants of exercise capacity (age and sex corrected peak VO_2) in AS.
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39 4. Determinants of MPR in AS.
- 40
41 5. Predictors of symptom development in AS.
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43 6. Predictors of progression of diffuse fibrosis and LV remodelling in AS at 12 months.
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45 7. Predictors of progression in microvascular dysfunction (reduced MPR) in AS at 12
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47 months.
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51 8. Reproducibility of MPR measurement in AS (sub-study).
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Recruitment and Data Collection (see figure 1)

Patients will be recruited from a number of regional hospitals, with testing performed at one of five tertiary cardiac centres in the UK with expertise in the management of AS and in CMR (Leicester, Leeds, Glasgow, Dundee and Aberdeen). Patients will be identified from cardiology clinics, echocardiography and MRI reports. An electronic case report form (e-CRF) will be used to collect study data, which has been developed by the Robertson Centre for Biostatistics, University of Glasgow. Access to the e-CRF will be restricted, with only authorised personnel able to make entries or amendments to patients' data.

Baseline Assessment

Written, informed-consent will be taken for all patients. Heart rate and blood pressure (BP) will be recorded and a resting ECG performed.

Venepuncture

A blood sample for clinical blood tests, including Haematocrit for calculation of myocardial extracellular volume (ECV), will be drawn. An additional 20 ml of venous blood will be collected for biomarkers. These samples will be immediately transferred on ice to a centrifuge, where they will be centrifuged at 2000 revolutions per minute, for 20 minutes, at 4°C. Once separated, the plasma will be pipetted into cryotubes in aliquots and stored in a cryobox in an electronically monitored freezer at -80°C, for analysis at the end of the study.

Biobanking

With additional consent, a blood sample will be drawn and banked for prospective research studies. All tissue will be collected, stored and disposed of in accordance with the Codes of Practice as laid out by the Human Tissue Authority.

Echocardiography

This will be undertaken according to the American Society of Echocardiography recommendations to determine AS severity and grade diastolic dysfunction[36]. At peak exercise, maximal peak and mean aortic valve velocity will be measured, allowing calculation of valve compliance[37].

Cardio-pulmonary Exercise Test

A symptom-limited maximal cardiopulmonary exercise test will be performed on a bicycle ergometer with workload increasing at 1-minute intervals. Workload increments will be based on patient age, gender, height and weight.[38] The test will be physician supervised and BP will be recorded at 2-minute intervals. Indications for medical termination will be as previously published[37]. Prior to the test initiation patients will be read the following statement: "Breathlessness is laboured or difficult breathing characterized by air hunger and an uncomfortable awareness of one's own breathing." The test will be considered symptomatically positive if the patient stops prematurely due to limiting breathlessness, chest tightness or dizziness at <80% of predicted workload. Results of the cardiopulmonary exercise test will not be reported unless the responsible Cardiologist would have performed an exercise test for clinical purposes.

Cardiac Magnetic Resonance

Patients will be imaged on 3T CMR platforms because of the better signal intensity and limits of agreement of myocardial blood flow with microspheres and better tag persistence compared to 1.5T, with similar LV function analysis. A comprehensive adenosine stress (140 micrograms/kg/min for 3 minutes) and rest perfusion study will be undertaken (Figure 2), to determine: 1. LV mass and volumes / Ejection Fraction. 2. Rest and stress myocardial blood flow and MPR. 3. LGE for focal fibrosis. 4. Pre and post contrast T1 mapping at a mid ventricular level 5. Tagging in three short axis slices.

CMR Analysis: The epicardium and endocardium will be contoured on the perfusion images, along with a region of interest in the LV blood pool, to generate signal intensity curves. The arterial input function corrected for signal saturation will be used for MBF quantification by model-independent deconvolution[39]. Transmural MPR will be calculated by dividing hyperemic blood flow by resting blood flow. Ten patients will undergo repeat adenosine stress CMR (within 10 days) to assess the reproducibility (coefficient of variation) of MPR in AS. Focal and diffuse fibrosis will be assessed using LGE and pre and post-contrast T1 mapping to estimate the myocardial ECV[40]. Tagging will be analysed using InTag post-processing toolbox (Creatix, Lyon, France) in OsiriX (Geneva, Switzerland).

At the 12-month visit, with additional consent, the rate of change in MPR will be assessed in those patients who have not developed symptoms. This will allow correlation of MPR with LV remodelling, diffuse and focal LV fibrosis development. All CMR scans will be analysed at the core lab (University of Leicester) by a single investigator blinded to all patient details.

CT Coronary Artery Calcium scoring (CTCAC)

The role of subclinical coronary artery disease in the progression to symptoms in asymptomatic AS is unclear. CTCAC will be performed on a multi-detector CT scanner with ECG gating, in a single breath-hold. Coronary artery calcification will be reported as present/absent and scored according to standard criteria to allow correlation of subclinical atherosclerosis in relation to MPR. CT scans will be analysed at the core lab in Leicester by a specialist Cardiac Radiologist. Reports will remain blinded except in the event of potentially life-threatening incidental findings and CTCAC >3 standard deviations above age predicted values. The extent of AV calcification will also be assessed in relation to valve compliance and clinical outcome.

Follow-Up

Patients will be followed up at 6 monthly intervals to a maximum of 30 months. Each visit will include a history on development of any typical symptoms, admissions to hospital, pre-specified MACE and venepuncture for NT-proBNP. For patients who report typical symptoms or MACE, the responsible clinician will be notified to consider referral for AVR. The 12-month visit will take place at the tertiary cardiac centres and if patients are asymptomatic, they will be invited to have repeat CMR as per baseline.

Event adjudication

All patients will be registered with the NHS Information service or the Information Services division in Scotland to verify outcomes and acquire long-term data. Two

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3 independent cardiologists will judge clinical events for the primary outcome.

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5 Disagreement will be resolved by consensus and if necessary by a third independent
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7 clinician.
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10 11 12 **Statistical analysis**

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15 This will be performed under the supervision of Prof Ford at the Robertson Centre for
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17 Biostatistics. All patients recruited will have a minimum of 1 year of follow-up for the
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19 primary outcome. The relationship between MPR and exercise testing with 1-year
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21 outcome will be analysed using logistic regression. The MPR cut-point for predicting
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23 the primary outcome will be determined from ROC analysis and will be selected to
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25 match the sensitivity of exercise-induced symptoms. Paired comparisons of the
26
27 specificities of the two approaches on the same dataset will be carried out using
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29 McNemar's test. The prognostic value of exercise test symptoms at low workload and
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31 MPR individually and in combination will be assessed for the full follow-up period
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33 using Cox-regression analyses, testing the significance of MPR in the presence of
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35 exercise test data, NT-proBNP and by calculating and comparing c-statistics
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37 (discrimination), Hosmer Lemeshow statistics (calibration) and net reclassification
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39 indices (prognostic value). Predictors of MPR and VO_2 will be assessed by univariate
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41 and multivariate regression analysis. Time to event data will be displayed using
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43 Kaplan-Meier curves and all model assumptions will be assessed using appropriate
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45 methods.
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Power Calculation

The study, with 170 subjects will have 80% power (binomial test) to show that MPR has superior overall accuracy (assumed 85%) in predicting symptom onset, compared to the results of previous studies for exercise testing (76%), assuming an annual event rate of 29%[15].

ETHICS AND DISSEMINATION

Ethics

The trial has full ethical approval from the National Research Ethics Service (NRES) Committee East Midlands (REC reference 11/EM/0410). It will be conducted according to the principles of the Medical Research Council Good Clinical Practice guidelines, Declaration of Helsinki, Data Protection Act, NHS Research Governance and relevant local and national laws. All patients will give written informed consent.

Study Organisation and Oversight

The Sponsor is the University Hospitals of Leicester NHS Trust. A Trial Steering Committee (TSC) has been appointed and is responsible for the scientific and ethical conduct of the study. This consists of the an Independent chairman and two members, the Chief Investigator, two co-investigators from the tertiary centres, a lay representative, representative of the Clinical Trials and Evaluation Unit and a representative of the Sponsor. The trial protocol and subsequent amendments have been approved by the TSC. A data monitoring committee was deemed not necessary given the observational design.

Study Timetable

Ethics application was approved in December 2011. Study enrolment started in April 2012 and recruitment is expected to be completed in November 2013 with a further 12 months for follow-up, post-processing and close-out of the study. The main study paper will be submitted within six months of study close-out.

FUNDING SOURCES

The study has been funded by a grant from the National Institute of Health Research, (NIHR) (Grant award number: NIHR-PDF 2011-04-51 Gerald P McCann). Additional support and resources for the trial will be provided by the NIHR Leicester Cardiovascular Biomedical Research Unit and NIHR Comprehensive Local Research Networks.

DISCUSSION

Limitations

The primary endpoint includes AVR for symptom development, which is subjective in nature. However, AVR by itself is deliberately not considered an endpoint due to the variability in clinical practice in referring severe but asymptomatic patients for surgery. Such patients will also be excluded from primary endpoint analysis. The presence of underlying coronary artery disease (CAD) may affect MPR and possibly symptom development. Although previous CABG / MI with 6 months is an exclusion criteria, the patients will not undergo coronary angiography to exclude CAD. The possibility of CT coronary angiography was considered but the high radiation dose at

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3 the time of study planning, administration of intravenous contrast and beta-blockers,
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5 was not felt to be justified in asymptomatic patients.
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10 **Anticipated Health Benefits**

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12 The study will address a number of limitations in previously published data, with the
13 primary endpoint being driven by symptom development and MACE. The study
14 should identify the strongest prognostic markers on which to base identification of
15 asymptomatic patients with moderate to severe AS for consideration of early (before
16 symptoms develop) AVR. The efficacy of such a strategy should be assessed in a
17 prospective randomised controlled trial of early surgery in those with impaired MPR
18 versus watchful waiting until symptom development.
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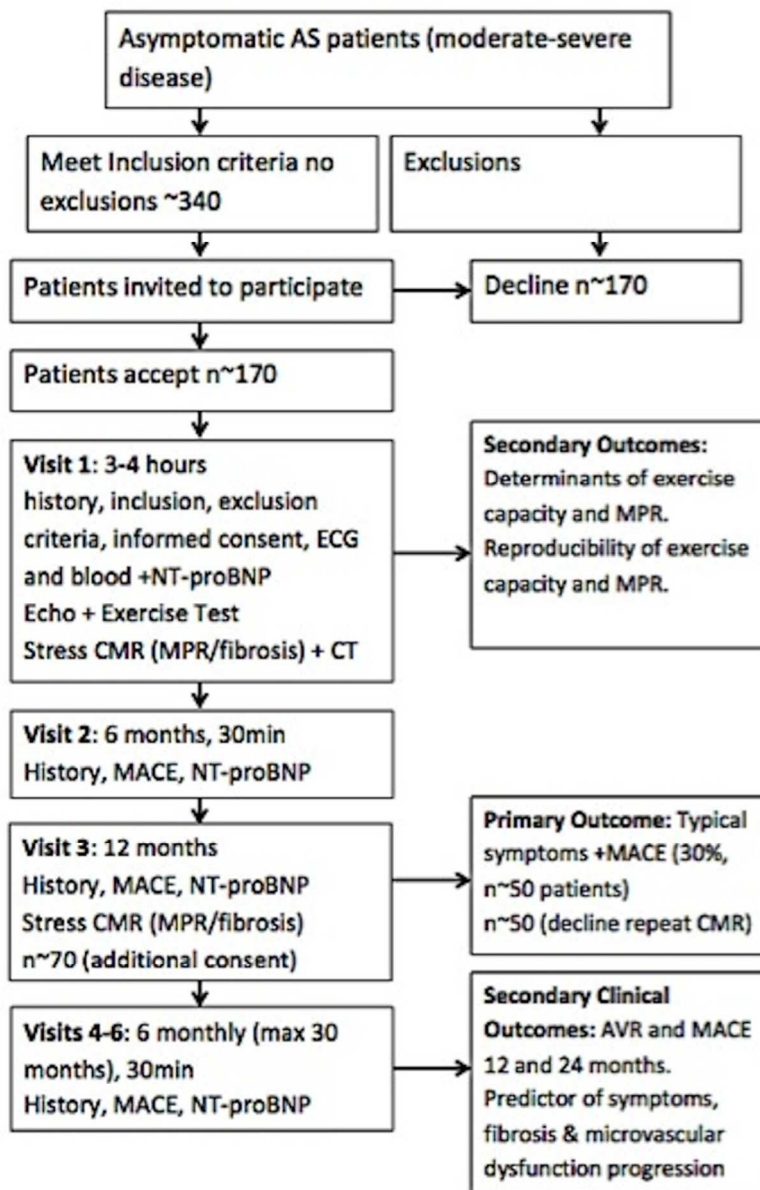
FIGURE LEGENDS

Figure 1: Flowchart demonstrating study plan

Figure 2: MRI Protocol used (Abbreviations- 4/2/3C: 4/2/3 chamber, LV: left ventricular, LA: left atrial, LVOT: left ventricular outflow tract; LGE: late gadolinium enhancement)

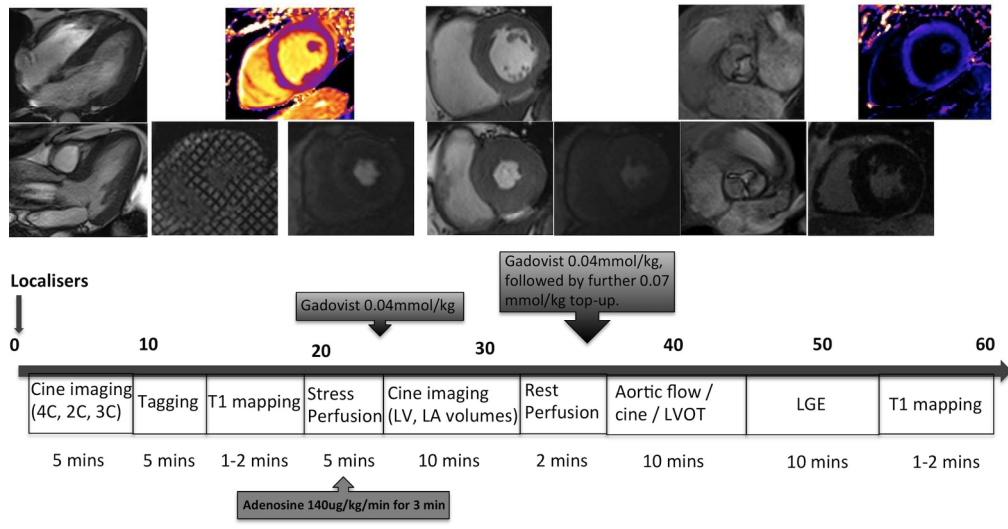
AUTHORS' CONTRIBUTIONS

AS is responsible for acquisition, analysis and interpretation of data and drafted the manuscript. IF and MJH are responsible for analysis and interpretation of data and critical revision of the article for important intellectual content. JPG, CB, SN, BP, BW and NJS were involved in conception and design of the study and revising the manuscript critically for important intellectual content. JNK and AU were involved in acquisition of data and revising the manuscript critically for important intellectual content. GPM is responsible for the conception and design of the study, drafting and critically revising the manuscript for intellectual content. All authors gave final approval of the manuscript.



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Review only



**Rationale and design of the PRognostic Importance of
MIcrovascular Dysfunction in asymptomatic patients with
Aortic Stenosis (PRIMID-AS)- a multicentre observational
study with blinded Investigations**

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Manuscripts

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4 **Rationale and design of the PRognostic Importance of MIcrovascular**
5 **Dysfunction in asymptomatic patients with Aortic Stenosis (PRIMID-AS)- a**
6 **Multicentre observational study with blinded Investigations**
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ABSTRACT

Introduction: Aortic stenosis (AS) is the commonest valve disorder in the developed world requiring surgery. Surgery in patients with severe asymptomatic AS remains controversial. Exercise testing can identify asymptomatic patients at increased risk of death and symptom development, but with limited specificity, especially in older adults. Cardiac Magnetic Resonance Imaging (CMR), including Myocardial Perfusion Reserve (MPR) may be a novel imaging biomarker in AS.

Aims: 1. To improve risk stratification in asymptomatic patients with AS, 2. To determine whether MPR is a better predictor of outcome than exercise testing and BNP.

Method/Design: Multi-centre, prospective observational study in the UK, comparing MPR with exercise testing and BNP (with blinded CMR analysis) for predicting outcome. **Population:** 170 asymptomatic patients with moderate-severe AS, who would be considered for Aortic Valve Replacement (AVR). **Primary outcome:** Composite of: typical symptoms necessitating referral for AVR and major adverse cardiovascular events. **Follow-up:** 12-30 months (minimum 12 months). **Primary Hypothesis:** MPR will be a better predictor of outcome than exercise testing and BNP.

Ethics / Dissemination: The study has full ethical approval and is actively recruiting patients. Data collection will be completed in November 2014 and the study results will be submitted for publication within 6 months of completion.

ClinicalTrials.gov identifier: NCT01658345.

STRENGTHS

- Study design: blinded imaging with core lab analysis (CMR and exercise testing) to reduce referral bias, inclusion only of patients who would consider surgery, multicentre, data collection/analysis by independent clinical trials unit.
- Comprehensive phenotyping of participants
- Management and oversight: Independent chair and members of steering committee, independent event adjudication
- Harder primary endpoint: asymptomatic patients referred for aortic valve replacement not included
- Registration with NHS Information services to minimise loss to follow-up and acquire long-term data

LIMITATIONS

- Coronary disease not excluded by angiography.
- CMR not widely available in all secondary care settings

INTRODUCTION

Aortic Stenosis (AS) is the commonest valve lesion requiring surgery in the developed world[1]. European registry data indicate that as few as 30% of potentially eligible patients are referred for surgery[2]. As the population in developed countries continues to age, it is predicted that the prevalence of AS will double in the next 20 years[3].

The development of symptoms in AS heralds a malignant phase of the condition and prompt AVR results in a clear reduction in mortality[4]. Surgery in this situation is universally regarded as a class I indication despite the absence of randomised controlled trials[5, 6]. In contrast, management of patients with severe AS in the absence of symptoms remains controversial and continues to provoke debate, with divergent clinical practice[7, 8]. It is generally agreed that the ideal timing of surgical intervention is immediately before a patient develops symptoms. Such a strategy, if successfully implemented, would minimise the risk of sudden death during the long latent (asymptomatic) period and minimise the number of patients sent for surgery who may never have developed symptoms related to AS.

Risk stratification in asymptomatic patients with moderate-severe AS

Many researchers have therefore sought clinical markers in asymptomatic patients with AS, that reliably identify those who will need AVR. The current body of evidence in asymptomatic AS consists mainly of observational studies (Table-1), which have identified a number of risk factors for developing symptoms or death[8,

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3 9]. These include a.) echocardiographic markers: aortic valve calcification, rapid
4 increase in pressure gradient[10], very high aortic valve velocities[11, 12]; b.) an
5 abnormal response or symptoms on exercise testing[13-15]; and c.) abnormal
6 biomarkers, especially Brain Natriuretic Peptides (BNP)[16]. It should be noted
7 however, that echocardiographic measures of AS severity are a poor discriminator
8 between those who go on to develop symptoms and those that do not, compared to
9 other parameters [13, 17].
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22 **Limitations of current research in asymptomatic AS**

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24 The limitations of these research studies have been highlighted in a recent editorial
25 [9]. These include a degree of selection bias due to non-randomisation, unblinded
26 investigations influencing management decisions and patients who subsequently
27 refuse surgery and patients undergoing AVR whilst asymptomatic being included in
28 the primary outcome.
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40 **Exercise testing to risk stratify in AS**

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42 Of the prognostic markers studied to date, most attention has focused on exercise
43 testing[13-15]. A recent meta-analysis of stress testing in 491 asymptomatic patients
44 with severe AS demonstrated that there was no sudden death in those with a normal
45 test, which was also associated with a low risk of subsequent events[18]. However
46 the specificity of a positive test is low for predicting outcome. Das et al showed the
47 positive predictive value of exercise symptoms to be only 57% and even lower in the
48 large and important group of patients >70 years of age[15]. It does seem likely that
49 the mechanisms limiting exercise capacity will be closely related to those that cause
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3 symptoms. However, no trial to date has assessed whether early AVR can improve
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5 outcome in asymptomatic patients with a positive exercise test (or for any other risk
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7 factor). For this reason the ACC/AHA guidelines grade exercise-induced symptoms
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9 as a class IIB indication (can be considered but weight of evidence does not support
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11 intervention) for AVR[19]. Exercise testing has not been widely implemented in
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13 clinical practice.[2]
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20 **Importance of Left Ventricular (LV) remodeling in AS**

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22 The hypertrophic response to pressure overload in severe AS is extremely variable[20,
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24 21]. When left ventricular hypertrophy (LVH) does occur, it is associated with a
25
26 number of detrimental pathophysiological sequelae: reduction in myocardial strain
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28 [22], development of myocardial fibrosis[23] and diastolic dysfunction. These
29
30 changes are also associated with microvascular dysfunction[24]. AS patients with
31
32 high left ventricular mass index (LVMI) are more likely to develop heart failure,
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34 suggesting that adverse ventricular remodeling is promotive of LV systolic
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36 dysfunction[21]. Inappropriately high LVMI has also been identified as an adverse
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38 prognostic marker in patients with severe asymptomatic AS[20].
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45 Following AVR there is regression of LVH which has been associated with
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47 improvement in myocardial strain[22], myocardial perfusion reserve[25] and exercise
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49 capacity[26], but not in all studies[27] However, there are concerns that LVH
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51 regression is incomplete and that myocardial fibrosis may be irreversible leading to
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53 persistent diastolic dysfunction and increased long-term mortality[28].
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Table 1: Prospective studies assessing risk stratification in moderate-severe asymptomatic AS

Author	No.	Severity	CAD	Outcome	Follow-up: months	AVR	Total/ Cardiac Deaths	SCD	Outcome predictor
Rosenhek [10]	128	Severe (>4m/s)	Not excluded	Death, AVR	22±18	59 of 106 (56%);	8 6 cardiac	1 (0.9%)	Calcification, rapid progression
Amato [13]	66	Severe (AVA <1cm ²)	Excluded (angio)	Death, symptoms	15 ±12	?34	4	4 (6.1%)	AVA < 0.7cm ² , positive ETT
Lancellotti [14]	69	Severe (AVA <1cm ²)	Not excluded,	Symptoms, death, AVR	15± 7	12 (17%),	3 cardiac + 1 death post AVR	2 (2.9%) SCD	Exercise mean PG +ve ETT, AVA< 0.75cm ²
Das [15]	125	Moderate to severe(AVA <1.4cm ²),	Not excluded,	Symptoms, death	12	36 (29%) symptoms ? AVR	No deaths	No SCD	Exercise symptoms
Monin [29]	104	Moderate-severe:>3m/s AVA <1.5cm	RWMA excluded	Indication for AVR, death	24	58 AVR	4 deaths (1 post AVR)		Female sex, BNP, peak velocity
Rosenhek [12]	116	Very severe >5m/s	No.	Indication for AVR, death	41 (median)	79 AVR, 10 refused AVR	17 deaths 9 no surgery 8 post AVR	1 SCD	Peak AV >5.5m/s, diabetes, Cholesterol
Kang [11]	197	Very severe >4.5m/s or AVA < 0.75cm ²	History or RWMA	Death	42 AVR 58 medical	148 102 early, 46 (of 95) medical	3 (0 cardiac) early, 28 (12 cardiac) medical	9 (10%) medical 0 early	Peak AV >5m/s
Cioffi [20]	209	Severe (AVA<1cm ² or mean PG>40 mmHg	History	Death, AVR, MI, HF hospitalisation	22 ± 13	72	20 (16 cardiac)	2 SCD	Inappropriate high LVMI, peak velocity, calcification

Abbreviations: AVA=aortic valve area; PG= pressure gradient; RWMA= regional wall motion abnormalities; AVR= aortic valve replacement; MI= myocardial infarction; HF= heart failure; AV= Aortic valve velocity; BNP= brain natriuretic peptide, angio= coronary angiography; LVMI= left ventricular mass index

(Adapted from [9]-reproduced with permission)

CMR in AS

Focal myocardial fibrosis can be detected non-invasively by late gadolinium enhancement (LGE) in 27-62% of AS patients[30, 31]. The extent of LGE correlates well with, although underestimates the extent of interstitial fibrosis on myocardial biopsy[30, 32] and increases with LVH[30, 31]. LGE in patients with severe AS is associated with limited improvement in symptoms, LV function and higher medium term mortality after AVR.[30, 32]

CMR myocardial tissue tagging can also demonstrate alterations in strain and strain rates, and is considered the gold standard technique for the assessment of function[33]. In AS there is an increase in the normal wringing action (torsion) of the LV, which improves after AVR[34]. The rate of untwisting in AS is reduced which may reduce diastolic filling and myocardial perfusion reserve (MPR) by loss of 'suction' action.

The importance of CMR detected LVH, LGE and MPR in predicting objectively measured maximal aerobic exercise capacity (peak VO_2) in 46 patients with severe isolated AS prior to AVR has been studied[35]. On stepwise regression analysis, MPR was the only independent predictor of sex and age-corrected peak oxygen consumption (peak VO_2), $\beta=0.457$, $p=0.001$. MPR also significantly decreased with increasing New York Heart Association (NYHA) Class ($p=0.001$). Newer CMR methods utilising T1 mapping also offer the possibility of quantifying diffuse myocardial fibrosis not detected by the LGE technique[26]. This has also been shown to be associated with exercise capacity and NYHA class in patients with severe AS prior to AVR[26]. Diffuse fibrosis may be an important determinant of MPR pre-

operatively. However early after AVR there is no significant change in diffuse fibrosis[26, 28] but MPR does increase[25] (reflecting changes in pressure overload and reverse LV remodeling[35]).

MPR is an attractive biomarker in AS since it is dependent on a combination of factors that include: valve severity and measures of LV remodelling/fibrosis and perfusion time. The primary aim of the PRIMID AS study is to assess whether MPR (and other CMR measures) can improve risk stratification in asymptomatic patients with moderate to severe AS, by comparing them to the best studied prognostic indicators: exercise testing and NT-proBNP.

STUDY DESIGN

This is a multi-centre, prospective observational study with blinded analysis of CMR data. The study has been registered on ClinicalTrials.gov (NCT01658345).

Aims of the Study

- To improve risk stratification in asymptomatic patients with AS.
- To determine whether MPR is a better predictor of outcome than exercise testing and NT-proBNP.
- To establish the determinants of MPR in asymptomatic AS.

Two substudies will:

- assess the reproducibility of MPR measurement in AS.

- assess the rate of progression in LV remodelling at 1 year in asymptomatic AS.

Primary Hypothesis

MPR will be a better predictor of adverse outcome than exercise testing in asymptomatic patients with moderate-severe AS.

Inclusion and Exclusion Criteria

These are shown in Table 2.

Table 2: Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Moderate-severe AS (≥ 2 of: AVA $< 1.5\text{cm}^2$, peak pressure gradient $> 36\text{mmHg}$, mean PG $> 25\text{mmHg}$).	History of CABG or recent MI within 6 months.
Asymptomatic.	Previous valve surgery.
Age > 18 years and < 85 years.	Severe valve disease other than AS.
Prepared to undergo AVR if symptoms develop.	Persistent Atrial fibrillation or flutter.
Ability to perform bicycle exercise test.	Severe Asthma.
	History of Heart Failure.
	Severe renal impairment eGFR $< 30\text{ml/min}$.
	Planned AVR.
	EF $< 40\%$.
	Any absolute contraindication to CMR.
	Contraindication to Adenosine.
	Other medical condition that limits life expectancy or precludes AVR
	Pregnancy.

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3 In addition, 20 asymptomatic controls without known cardiac disease will undergo
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5 baseline assessment to allow determination of age and sex-matched normal ranges for
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7 MPR, diffuse myocardial fibrosis and exercise capacity.
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10 11 12 13 **Primary Outcome Measures**

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15 Composite of typical AS symptoms necessitating referral for AVR, cardiovascular
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17 death and Major Adverse Cardiovascular Events (MACE) (hospitalisation with any of:
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19 heart failure, chest pain, syncope, arrhythmia or stroke) at 12 months (time to first
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21 event). Asymptomatic patients having AVR for other reasons (valve progression,
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23 positive exercise test) will be excluded from primary endpoint analysis.
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31 **Secondary Outcome Measures**

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33 1. Composite of AVR (for any reason) and MACE at 12 months (first event).
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35 2. Composite of AVR and MACE during follow-up (first event).
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37 3. Determinants of exercise capacity (age and sex corrected peak VO_2) in AS.
- 38
39 4. Determinants of MPR in AS.
- 40
41 5. Predictors of symptom development in AS.
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43 6. Predictors of progression of diffuse fibrosis and LV remodelling in AS at 12 months.
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45 7. Predictors of progression in microvascular dysfunction (reduced MPR) in AS at 12
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47 months.
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51 8. Reproducibility of MPR measurement in AS (sub-study).
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Recruitment and Data Collection (see figure 1)

Patients will be recruited from a number of regional hospitals, with testing performed at one of five tertiary cardiac centres in the UK with expertise in the management of AS and in CMR (Leicester, Leeds, Glasgow, Dundee and Aberdeen). Patients will be identified from cardiology clinics, echocardiography and MRI reports. Suitable patients will be approached in outpatient clinics either by a member of the clinical team and given a patient information sheet (PIS) if interested. Those who are not due in clinic in the near future will be posted a PIS with a reply form and stamped addressed envelope. An electronic case report form (e-CRF) will be used to collect study data, which has been developed by the Robertson Centre for Biostatistics, University of Glasgow. Access to the e-CRF will be restricted, with only authorised personnel able to make entries or amendments to patients' data.

Baseline Assessment

Written, informed-consent will be taken for all patients. Heart rate and blood pressure (BP) will be recorded and a resting ECG performed.

Venepuncture

A blood sample for clinical blood tests, including Haematocrit for calculation of myocardial extracellular volume (ECV), will be drawn. An additional 20 ml of venous blood will be collected for biomarkers. These samples will be immediately transferred on ice to a centrifuge, where they will be centrifuged at 2000 revolutions per minute, for 20 minutes, at 4°C. Once separated, the plasma will be pipetted into cryotubes in aliquots and stored in a cryobox in an electronically monitored freezer at -80°C, for analysis at the end of the study.

Biobanking

With additional consent, a blood sample will be drawn and banked for prospective research studies. All tissue will be collected, stored and disposed of in accordance with the Codes of Practice as laid out by the Human Tissue Authority.

Echocardiography

This will be undertaken according to the American Society of Echocardiography recommendations to determine AS severity and grade diastolic dysfunction[36]. At peak exercise, maximal peak and mean aortic valve velocity will be measured, allowing calculation of valve compliance[37].

Cardio-pulmonary Exercise Test

A symptom-limited maximal cardiopulmonary exercise test will be performed on a bicycle ergometer with workload increasing at 1-minute intervals. Workload increments will be based on patient age, gender, height and weight.[38] The test will be physician supervised and BP will be recorded at 2-minute intervals. Indications for medical termination will be as previously published[37]. Prior to the test initiation patients will be read the following statement: "Breathlessness is laboured or difficult breathing characterized by air hunger and an uncomfortable awareness of one's own breathing." The test will be considered symptomatically positive if the patient stops prematurely due to limiting breathlessness, chest tightness or dizziness at <80% of predicted workload. Results of the cardiopulmonary exercise test will not be reported unless the responsible Cardiologist would have performed an exercise test for clinical purposes.

Cardiac Magnetic Resonance

Patients will be imaged on 3T CMR platforms because of the better signal intensity and limits of agreement of myocardial blood flow with microspheres and better tag persistence compared to 1.5T, with similar LV function analysis. A comprehensive adenosine stress (140 micrograms/kg/min for 3 minutes) and rest perfusion study will be undertaken (Figure 2), to determine: 1. LV mass and volumes / Ejection Fraction. 2. Rest and stress myocardial blood flow and MPR. 3. LGE for focal fibrosis. 4. Pre and post contrast T1 mapping at a mid ventricular level 5. Tagging in three short axis slices.

CMR Analysis: The epicardium and endocardium will be contoured on the perfusion images, along with a region of interest in the LV blood pool, to generate signal intensity curves. The arterial input function corrected for signal saturation will be used for MBF quantification by model-independent deconvolution[39]. Transmural MPR will be calculated by dividing hyperemic blood flow by resting blood flow. Ten patients will undergo repeat adenosine stress CMR (within 10 days) to assess the reproducibility (coefficient of variation) of MPR in AS. Focal and diffuse fibrosis will be assessed using LGE and pre and post-contrast T1 mapping to estimate the myocardial ECV[40]. Tagging will be analysed using InTag post-processing toolbox (Creatix, Lyon, France) in OsiriX (Geneva, Switzerland).

At the 12-month visit, with additional consent, the rate of change in MPR will be assessed in those patients who have not developed symptoms. This will allow correlation of MPR with LV remodelling, diffuse and focal LV fibrosis development. All CMR scans will be analysed at the core lab (University of Leicester) by a single investigator blinded to all patient details.

CT Coronary Artery Calcium scoring (CTCAC)

The role of subclinical coronary artery disease in the progression to symptoms in asymptomatic AS is unclear. CTCAC will be performed on a multi-detector CT scanner with ECG gating, in a single breath-hold. Coronary artery calcification will be reported as present/absent and scored according to standard criteria to allow correlation of subclinical atherosclerosis in relation to MPR. CT scans will be analysed at the core lab in Leicester by a specialist Cardiac Radiologist. Reports will remain blinded except in the event of potentially life-threatening incidental findings and CTCAC >3 standard deviations above age predicted values. The extent of AV calcification will also be assessed in relation to valve compliance and clinical outcome.

Follow-Up

Patients will be followed up at 6 monthly intervals to a maximum of 30 months. The research team will contact patients by telephone just before the follow-up appointment is due, in order to optimise attendance, and travel costs will be covered for their follow-up visits if required. Each visit will include a history on development of any typical symptoms, admissions to hospital, pre-specified MACE and venepuncture for NT-proBNP. For patients who report typical symptoms or MACE, the responsible clinician will be notified to consider referral for AVR. The 12-month visit will take place at the tertiary cardiac centres and if patients are asymptomatic, they will be invited to have repeat CMR as per baseline.

Event adjudication

All patients will be registered with the NHS Information service or the Information Services division in Scotland to verify outcomes and acquire long-term data. Two independent cardiologists will judge clinical events for the primary outcome. Disagreement will be resolved by consensus and if necessary by a third independent clinician.

Statistical analysis

This will be performed under the supervision of Prof Ford at the Robertson Centre for Biostatistics. All patients recruited will have a minimum of 1 year of follow-up for the primary outcome. The relationship between MPR and exercise testing with 1-year outcome will be analysed using logistic regression. The MPR cut-point for predicting the primary outcome will be determined from ROC analysis and will be selected to match the sensitivity of exercise-induced symptoms. Paired comparisons of the specificities of the two approaches on the same dataset will be carried out using McNemar's test. The prognostic value of exercise test symptoms at low workload and MPR individually and in combination will be assessed for the full follow-up period using Cox-regression analyses, testing the significance of MPR in the presence of exercise test data, NT-proBNP and by calculating and comparing c-statistics (discrimination), Hosmer Lemeshow statistics (calibration) and net reclassification indices (prognostic value). Predictors of MPR and VO_2 will be assessed by univariate and multivariate regression analysis. Time to event data will be displayed using Kaplan-Meier curves and all model assumptions will be assessed using appropriate methods.

Power Calculation

The study, with 170 subjects will have 80% power (binomial test) to show that MPR has superior overall accuracy (assumed 85%) in predicting symptom onset, compared to the results of previous studies for exercise testing (76%), assuming an annual event rate of 29%[15].

ETHICS AND DISSEMINATION

Ethics

The study has full ethical approval from the National Research Ethics Service (NRES) Committee East Midlands (REC reference 11/EM/0410). It will be conducted according to the principles of the Medical Research Council Good Clinical Practice guidelines, Declaration of Helsinki, Data Protection Act, NHS Research Governance and relevant local and national laws. All patients will provide written informed consent during their first visit, before any tests are carried out, and will have had at least 24 hours to decide whether to participate or not prior to this.

Study Organisation and Oversight

The Sponsor is the University Hospitals of Leicester NHS Trust. A Trial Steering Committee (TSC) has been appointed and is responsible for the scientific and ethical conduct of the study. This consists of the an Independent chairman and two members, the Chief Investigator, two co-investigators from the tertiary centres, a lay representative, representative of the Clinical Trials and Evaluation Unit and a

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3 representative of the Sponsor. The study protocol and subsequent amendments have
4
5 been approved by the TSC. A data monitoring committee was deemed not necessary
6
7 given the observational design.
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10 11 **Study Timetable**

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13 Ethics application was approved in December 2011. Study enrolment started in April
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15 2012 and recruitment is expected to be completed in November 2013 with a further 12
16
17 months for follow-up, post-processing and close-out of the study. The main study paper
18
19 will be submitted within six months of study close-out.
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26 **FUNDING SOURCES**

27
28 The study has been funded by a grant from the National Institute of Health Research,
29
30 (NIHR) (Grant award number: NIHR-PDF 2011-04-51 Gerald P McCann).
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32 Additional support and resources for the study will be provided by the NIHR
33
34 Leicester Cardiovascular Biomedical Research Unit and NIHR Comprehensive Local
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36 Research Networks.
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43 **DISCUSSION**

44 45 46 **Limitations**

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48 The primary endpoint includes AVR for symptom development, which is subjective
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50 in nature. However, AVR by itself is deliberately not considered an endpoint due to
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52 the variability in clinical practice in referring severe but asymptomatic patients for
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54 surgery. Such patients will also be excluded from primary endpoint analysis. The
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3 presence of underlying coronary artery disease (CAD) may affect MPR and possibly
4 symptom development. Although previous CABG / MI with 6 months is an exclusion
5 criteria, the patients will not undergo coronary angiography to exclude CAD. The
6 possibility of CT coronary angiography was considered but the high radiation dose at
7 the time of study planning, administration of intravenous contrast and beta-blockers,
8 was not felt to be justified in asymptomatic patients.
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20 **Anticipated Health Benefits**

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22 The study will address a number of limitations in previously published data, with the
23 primary endpoint being driven by symptom development and MACE. The study
24 should identify the strongest prognostic markers on which to base identification of
25 asymptomatic patients with moderate to severe AS for consideration of early (before
26 symptoms develop) AVR. The efficacy of such a strategy should be assessed in a
27 prospective randomised controlled trial of early surgery in those with impaired MPR
28 versus watchful waiting until symptom development.
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FIGURE LEGENDS

Figure 1: Flowchart demonstrating study plan

Figure 2: MRI Protocol used (Abbreviations- 4/2/3C: 4/2/3 chamber, LV: left ventricular, LA: left atrial, LVOT: left ventricular outflow tract; LGE: late gadolinium enhancement)

AUTHORS' CONTRIBUTIONS

AS is responsible for acquisition, analysis and interpretation of data and drafted the manuscript. IF and MJH are responsible for analysis and interpretation of data and critical revision of the article for important intellectual content. JPG, CB, SN, BP, BW and NJS were involved in conception and design of the study and revising the manuscript critically for important intellectual content. JNK and AU were involved in acquisition of data and revising the manuscript critically for important intellectual content. GPM is responsible for the conception and design of the study, drafting and critically revising the manuscript for intellectual content. All authors gave final approval of the manuscript.

COMPETING INTERESTS

There are no competing interests.

**Rationale and design of the PRognostic Importance of MIcrovascular
Dysfunction in asymptomatic patients with Aortic Stenosis (PRIMID-AS)- a
multicentre observational study with blinded Investigations**

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Key words: aortic stenosis, cardiac MRI, myocardial perfusion reserve, exercise
testing

Word count = ~~3103~~3085 (main text only)

ABSTRACT

Introduction: Aortic stenosis (AS) is the commonest valve disorder in the developed world requiring surgery. Surgery in patients with severe asymptomatic AS remains controversial. Exercise testing can identify asymptomatic patients at increased risk of death and symptom development, but with limited specificity, especially in older adults. Cardiac Magnetic Resonance Imaging (CMR), including Myocardial Perfusion Reserve (MPR) may be a novel imaging biomarker in AS.

Aims: 1. To improve risk stratification in asymptomatic patients with AS, 2. To determine whether MPR is a better predictor of outcome than exercise testing and BNP.

Method/Design: Multi-centre, prospective observational study in the UK, comparing MPR with exercise testing and BNP (with blinded CMR analysis) for predicting outcome. **Population:** 170 asymptomatic patients with moderate-severe AS, who would be considered for Aortic Valve Replacement (AVR). **Primary outcome:** Composite of: typical symptoms necessitating referral for AVR and major adverse cardiovascular events. **Follow-up:** 12-30 months (minimum 12 months). **Primary Hypothesis:** MPR will be a better predictor of outcome than exercise testing and BNP.

Ethics / Dissemination: The study has full ethical approval and is actively recruiting patients. Data collection will be completed in November 2014 and the study results will be submitted for publication within 6 months of completion.

ClinicalTrials.gov identifier: NCT01658345.

STRENGTHS

- Study design: blinded imaging with core lab analysis (CMR and exercise testing) to reduce referral bias, inclusion only of patients who would consider surgery, multicentre, data collection/analysis by independent clinical trials unit.
- Comprehensive phenotyping of participants
- Management and oversight: Independent chair and members of steering committee, independent event adjudication
- Harder primary endpoint: asymptomatic patients referred for aortic valve replacement not included
- Registration with NHS Information services to minimise loss to follow-up and acquire long-term data

LIMITATIONS

- Coronary disease not excluded by angiography.
- CMR not widely available in all secondary care settings

INTRODUCTION

Aortic Stenosis (AS) is the commonest valve lesion requiring surgery in the developed world[1]. European registry data indicate that as few as 30% of potentially eligible patients are referred for surgery[2]. As the population in developed countries continues to age, it is predicted that the prevalence of AS will double in the next 20 years[3].

The development of symptoms in AS heralds a malignant phase of the condition and prompt AVR results in a clear reduction in mortality[4]. Surgery in this situation is universally regarded as a class I indication despite the absence of randomised controlled trials[5, 6]. In contrast, management of patients with severe AS in the absence of symptoms remains controversial and continues to provoke debate, with divergent clinical practice[7, 8]. It is generally agreed that the ideal timing of surgical intervention is immediately before a patient develops symptoms. Such a strategy, if successfully implemented, would minimise the risk of sudden death during the long latent (asymptomatic) period and minimise the number of patients sent for surgery who may never have developed symptoms related to AS.

Risk stratification in asymptomatic patients with moderate-severe AS

Many researchers have therefore sought clinical markers in asymptomatic patients with AS, that reliably identify those who will need AVR. The current body of evidence in asymptomatic AS consists mainly of observational studies (Table-1), which have identified a number of risk factors for developing symptoms or death[8,

9]. These include a.) echocardiographic markers: aortic valve calcification, rapid increase in pressure gradient[10], very high aortic valve velocities[11, 12]; b.) an abnormal response or symptoms on exercise testing[13-15]; and c.) abnormal biomarkers, especially Brain Natriuretic Peptides (BNP)[16]. It should be noted however, that echocardiographic measures of AS severity are a poor discriminator between those who go on to develop symptoms and those that do not, compared to other parameters [13, 17].

Limitations of current research in asymptomatic AS

The limitations of these research studies have been highlighted in a recent editorial [9]. These include a degree of selection bias due to non-randomisation, unblinded investigations influencing management decisions and patients who subsequently refuse surgery and patients undergoing AVR whilst asymptomatic being included in the primary outcome.

Exercise testing to risk stratify in AS

Of the prognostic markers studied to date, most attention has focused on exercise testing[13-15]. A recent meta-analysis of stress testing in 491 asymptomatic patients with severe AS demonstrated that there was no sudden death in those with a normal test, which was also associated with a low risk of subsequent events[18]. However the specificity of a positive test is low for predicting outcome. Das et al showed the positive predictive value of exercise symptoms to be only 57% and even lower in the large and important group of patients >70 years of age[15]. It does seem likely that the mechanisms limiting exercise capacity will be closely related to those that cause

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6 symptoms. However, no trial to date has assessed whether early AVR can improve
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8 outcome in asymptomatic patients with a positive exercise test (or for any other risk
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10 factor). For this reason the ACC/AHA guidelines grade exercise-induced symptoms
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12 as a class IIB indication (can be considered but weight of evidence does not support
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14 intervention) for AVR[19]. Exercise testing has not been widely implemented in
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16 clinical practice.[2]
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21 **Importance of Left Ventricular (LV) remodeling in AS**

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23 The hypertrophic response to pressure overload in severe AS is extremely variable[20,
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25 21]. When left ventricular hypertrophy (LVH) does occur, it is associated with a
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27 number of detrimental pathophysiological sequelae: reduction in myocardial strain
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29 [22], development of myocardial fibrosis[23] and diastolic dysfunction. These
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31 changes are also associated with microvascular dysfunction[24]. AS patients with
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33 high left ventricular mass index (LVMI) are more likely to develop heart failure,
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35 suggesting that adverse ventricular remodeling is promotive of LV systolic
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37 dysfunction[21]. Inappropriately high LVMI has also been identified as an adverse
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39 prognostic marker in patients with severe asymptomatic AS[20].
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43 Following AVR there is regression of LVH which has been associated with
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45 improvement in myocardial strain[22], myocardial perfusion reserve[25] and exercise
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47 capacity[26], but not in all studies[27] However, there are concerns that LVH
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49 regression is incomplete and that myocardial fibrosis may be irreversible leading to
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51 persistent diastolic dysfunction and increased long-term mortality[28].
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Table 1: Prospective studies assessing risk stratification in moderate-severe asymptomatic AS

Author	No.	Severity	CAD	Outcome	Follow-up: months	AVR	Total/ Cardiac Deaths	SCD	Outcome predictor
Rosenhek [10]	128	Severe (>4m/s)	Not excluded	Death, AVR	22±18	59 of 106 (56%);	8 6 cardiac	1 (0.9%)	Calcification, rapid progression
Amato [13]	66	Severe (AVA <1cm ²)	Excluded (angio)	Death, symptoms	15±12	?34	4	4 (6.1%)	AVA <0.7cm ² , positive ETT
Lancellotti [14]	69	Severe (AVA <1cm ²)	Not excluded,	Symptoms, death, AVR	15± 7	12 (17%),	3 cardiac + 1 death post AVR	2 (2.9%) SCD	Exercise mean PG +ve ETT, AVA<0.75cm ²
Das [15]	125	Moderate to severe (AVA <1.4cm ²),	Not excluded,	Symptoms, death	12	36 (29%) symptoms ? AVR	No deaths	No SCD	Exercise symptoms
Monin [29]	104	Moderate-severe:>3m/s AVA <1.5cm	RWMA excluded	Indication for AVR, death	24	58 AVR	4 deaths (1 post AVR)		Female sex, BNP, peak velocity
Rosenhek [12]	116	Very severe >5m/s	No.	Indication for AVR, death	41 (median)	79 AVR, 10 refused AVR	17 deaths 9 no surgery 8 post AVR	1 SCD	Peak AV >5.5m/s, diabetes, Cholesterol
Kang [11]	197	Very severe >4.5m/s or AVA <0.75cm ²	History or RWMA	Death	42 AVR 58 medical	148 102 early, 46 (of 95) medical	3 (0 cardiac) early, 28 (12 cardiac) medical	9 (10%) medical 0 early	Peak AV >5m/s
Cioffi [20]	209	Severe (AVA<1cm ² or mean PG>40 mmHg)	History	Death, AVR, MI, HF hospitalisation	22 ± 13	72	20 (16 cardiac)	2 SCD	Inappropriate high LVMI, peak velocity, calcification

Abbreviations: AVA=aortic valve area; PG= pressure gradient; RWMA= regional wall motion abnormalities; AVR= aortic valve replacement; MI= myocardial infarction; HF= heart failure; AV= Aortic valve velocity; BNP= brain natriuretic peptide, angio= coronary angiography; LVMI= left ventricular mass index

(Adapted from [9]-reproduced with permission)

CMR in AS

Focal myocardial fibrosis can be detected non-invasively by late gadolinium enhancement (LGE) in 27-62% of AS patients[30, 31]. The extent of LGE correlates well with, although underestimates the extent of interstitial fibrosis on myocardial biopsy[30, 32] and increases with LVH[30, 31]. LGE in patients with severe AS is associated with limited improvement in symptoms, LV function and higher medium term mortality after AVR.[30, 32]

CMR myocardial tissue tagging can also demonstrate alterations in strain and strain rates, and is considered the gold standard technique for the assessment of function[33]. In AS there is an increase in the normal wringing action (torsion) of the LV, which improves after AVR[34]. The rate of untwisting in AS is reduced which may reduce diastolic filling and myocardial perfusion reserve (MPR) by loss of 'suction' action.

The importance of CMR detected LVH, LGE and MPR in predicting objectively measured maximal aerobic exercise capacity (peak VO_2) in 46 patients with severe isolated AS prior to AVR has been studied[35]. On stepwise regression analysis, MPR was the only independent predictor of sex and age-corrected peak oxygen consumption (peak VO_2), $\beta=0.457$, $p=0.001$. MPR also significantly decreased with increasing New York Heart Association (NYHA) Class ($p=0.001$). Newer CMR methods utilising T1 mapping also offer the possibility of quantifying diffuse myocardial fibrosis not detected by the LGE technique[26]. This has also been shown to be associated with exercise capacity and NYHA class in patients with severe AS prior to AVR[26]. Diffuse fibrosis may be an important determinant of MPR pre-

operatively. However early after AVR there is no significant change in diffuse fibrosis[26, 28] but MPR does increase[25] (reflecting changes in pressure overload and reverse LV remodeling[35]).

MPR is an attractive biomarker in AS since it is dependent on a combination of factors that include: valve severity and measures of LV remodelling/fibrosis and perfusion time. The primary aim of the PRIMID AS study is to assess whether MPR (and other CMR measures) can improve risk stratification in asymptomatic patients with moderate to severe AS, by comparing them to the best studied prognostic indicators: exercise testing and NT-proBNP.

STUDY DESIGN

This is a multi-centre, prospective ~~trial~~-observational study with blinded analysis of CMR data. The ~~trial~~-study has been registered on ClinicalTrials.gov (NCT01658345).

Aims of the Study

- To improve risk stratification in asymptomatic patients with AS.
- To determine whether MPR is a better predictor of outcome than exercise testing and NT-proBNP.
- To establish the determinants of MPR in asymptomatic AS.

Two substudies will:

- assess the reproducibility of MPR measurement in AS.

- assess the rate of progression in LV remodelling at 1 year in asymptomatic AS.

Primary Hypothesis

MPR will be a better predictor of adverse outcome than exercise testing in asymptomatic patients with moderate-severe AS.

Inclusion and Exclusion Criteria

These are shown in Table 2.

Table 2: Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Moderate-severe AS (≥ 2 of: AVA $< 1.5\text{cm}^2$, peak pressure gradient $> 36\text{mmHg}$, mean PG $> 25\text{mmHg}$).	History of CABG or recent MI within 6 months.
Asymptomatic.	Previous valve surgery.
Age > 18 years and < 85 years.	Severe valve disease other than AS.
Prepared to undergo AVR if symptoms develop.	Persistent Atrial fibrillation or flutter.
Ability to perform bicycle exercise test.	Severe Asthma.
	History of Heart Failure.
	Severe renal impairment eGFR $< 30\text{ml/min}$.
	Planned AVR.
	EF $< 40\%$.
	Any absolute contraindication to CMR.
	Contraindication to Adenosine.
	Other medical condition that limits life expectancy or precludes AVR
	Pregnancy.

In addition, 20 asymptomatic controls without known cardiac disease will undergo baseline assessment to allow determination of age and sex-matched normal ranges for MPR, diffuse myocardial fibrosis and exercise capacity.

Primary Outcome Measures

Composite of typical AS symptoms necessitating referral for AVR, cardiovascular death and Major Adverse Cardiovascular Events (MACE) (hospitalisation with any of: heart failure, chest pain, syncope, arrhythmia or stroke) at 12 months (time to first event). Asymptomatic patients having AVR for other reasons (valve progression, positive exercise test) will be excluded from primary endpoint analysis.

Secondary Outcome Measures

1. Composite of AVR (for any reason) and MACE at 12 months (first event).
2. Composite of AVR and MACE during follow-up (first event).
3. Determinants of exercise capacity (age and sex corrected peak VO_2) in AS.
4. Determinants of MPR in AS.
5. Predictors of symptom development in AS.
6. Predictors of progression of diffuse fibrosis and LV remodelling in AS at 12 months.
7. Predictors of progression in microvascular dysfunction (reduced MPR) in AS at 12 months.
8. Reproducibility of MPR measurement in AS (sub-study).

Recruitment and Data Collection (see figure 1)

Patients will be recruited from a number of regional hospitals, with testing performed at one of five tertiary cardiac centres in the UK with expertise in the management of AS and in CMR (Leicester, Leeds, Glasgow, Dundee and Aberdeen). Patients will be identified from cardiology clinics, echocardiography and MRI reports. Suitable patients will be approached in outpatient clinics either by a member of the clinical team and given a patient information sheet (PIS) if interested. Those who are not due in clinic in the near future will be posted a PIS with a reply form and stamped addressed envelope. An electronic case report form (e-CRF) will be used to collect study data, which has been developed by the Robertson Centre for Biostatistics, University of Glasgow. Access to the e-CRF will be restricted, with only authorised personnel able to make entries or amendments to patients' data.

Baseline Assessment

Written, informed-consent will be taken for all patients. Heart rate and blood pressure (BP) will be recorded and a resting ECG performed.

Venepuncture

A blood sample for clinical blood tests, including Haematocrit for calculation of myocardial extracellular volume (ECV), will be drawn. An additional 20 ml of venous blood will be collected for biomarkers. These samples will be immediately transferred on ice to a centrifuge, where they will be centrifuged at 2000 revolutions per minute, for 20 minutes, at 4°C. Once separated, the plasma will be pipetted into cryotubes in aliquots and stored in a cryobox in an electronically monitored freezer at -80°C, for analysis at the end of the study.

Biobanking

With additional consent, a blood sample will be drawn and banked for prospective research studies. All tissue will be collected, stored and disposed of in accordance with the Codes of Practice as laid out by the Human Tissue Authority.

Echocardiography

This will be undertaken according to the American Society of Echocardiography recommendations to determine AS severity and grade diastolic dysfunction[36]. At peak exercise, maximal peak and mean aortic valve velocity will be measured, allowing calculation of valve compliance[37].

Cardio-pulmonary Exercise Test

A symptom-limited maximal cardiopulmonary exercise test will be performed on a bicycle ergometer with workload increasing at 1-minute intervals. Workload increments will be based on patient age, gender, height and weight.[38] The test will be physician supervised and BP will be recorded at 2-minute intervals. Indications for medical termination will be as previously published[37]. Prior to the test initiation patients will be read the following statement: "Breathlessness is laboured or difficult breathing characterized by air hunger and an uncomfortable awareness of one's own breathing." The test will be considered symptomatically positive if the patient stops prematurely due to limiting breathlessness, chest tightness or dizziness at <80% of predicted workload. Results of the cardiopulmonary exercise test will not be reported unless the responsible Cardiologist would have performed an exercise test for clinical purposes.

Cardiac Magnetic Resonance

Patients will be imaged on 3T CMR platforms because of the better signal intensity and limits of agreement of myocardial blood flow with microspheres and better tag persistence compared to 1.5T, with similar LV function analysis. A comprehensive adenosine stress (140 micrograms/kg/min for 3 minutes) and rest perfusion study will be undertaken (Figure 2), to determine: 1. LV mass and volumes / Ejection Fraction. 2. Rest and stress myocardial blood flow and MPR. 3. LGE for focal fibrosis. 4. Pre and post contrast T1 mapping at a mid ventricular level 5. Tagging in three short axis slices.

CMR Analysis: The epicardium and endocardium will be contoured on the perfusion images, along with a region of interest in the LV blood pool, to generate signal intensity curves. The arterial input function corrected for signal saturation will be used for MBF quantification by model-independent deconvolution[39]. Transmural MPR will be calculated by dividing hyperemic blood flow by resting blood flow. Ten patients will undergo repeat adenosine stress CMR (within 10 days) to assess the reproducibility (coefficient of variation) of MPR in AS. Focal and diffuse fibrosis will be assessed using LGE and pre and post-contrast T1 mapping to estimate the myocardial ECV[40]. Tagging will be analysed using InTag post-processing toolbox (Creatis, Lyon, France) in OsiriX (Geneva, Switzerland).

At the 12-month visit, with additional consent, the rate of change in MPR will be assessed in those patients who have not developed symptoms. This will allow correlation of MPR with LV remodelling, diffuse and focal LV fibrosis development. All CMR scans will be analysed at the core lab (University of Leicester) by a single investigator blinded to all patient details.

CT Coronary Artery Calcium scoring (CTCAC)

The role of subclinical coronary artery disease in the progression to symptoms in asymptomatic AS is unclear. CTCAC will be performed on a multi-detector CT scanner with ECG gating, in a single breath-hold. Coronary artery calcification will be reported as present/absent and scored according to standard criteria to allow correlation of subclinical atherosclerosis in relation to MPR. CT scans will be analysed at the core lab in Leicester by a specialist Cardiac Radiologist. Reports will remain blinded except in the event of potentially life-threatening incidental findings and CTCAC >3 standard deviations above age predicted values. The extent of AV calcification will also be assessed in relation to valve compliance and clinical outcome.

Follow-Up

Patients will be followed up at 6 monthly intervals to a maximum of 30 months. The research team will contact patients by telephone just before the follow-up appointment is due, in order to optimise attendance, and travel costs will be covered for their follow-up visits if required. Each visit will include a history on development of any typical symptoms, admissions to hospital, pre-specified MACE and venepuncture for NT-proBNP. For patients who report typical symptoms or MACE, the responsible clinician will be notified to consider referral for AVR. The 12-month visit will take place at the tertiary cardiac centres and if patients are asymptomatic, they will be invited to have repeat CMR as per baseline.

Event adjudication

All patients will be registered with the NHS Information service or the Information Services division in Scotland to verify outcomes and acquire long-term data. Two independent cardiologists will judge clinical events for the primary outcome.

Disagreement will be resolved by consensus and if necessary by a third independent clinician.

Statistical analysis

This will be performed under the supervision of Prof Ford at the Robertson Centre for Biostatistics. All patients recruited will have a minimum of 1 year of follow-up for the primary outcome. The relationship between MPR and exercise testing with 1-year outcome will be analysed using logistic regression. The MPR cut-point for predicting the primary outcome will be determined from ROC analysis and will be selected to match the sensitivity of exercise-induced symptoms. Paired comparisons of the specificities of the two approaches on the same dataset will be carried out using McNemar's test. The prognostic value of exercise test symptoms at low workload and MPR individually and in combination will be assessed for the full follow-up period using Cox-regression analyses, testing the significance of MPR in the presence of exercise test data, NT-proBNP and by calculating and comparing c-statistics (discrimination), Hosmer Lemeshow statistics (calibration) and net reclassification indices (prognostic value). Predictors of MPR and VO_2 will be assessed by univariate and multivariate regression analysis. Time to event data will be displayed using Kaplan-Meier curves and all model assumptions will be assessed using appropriate methods.

Power Calculation

The study, with 170 subjects will have 80% power (binomial test) to show that MPR has superior overall accuracy (assumed 85%) in predicting symptom onset, compared to the results of previous studies for exercise testing (76%), assuming an annual event rate of 29%[15].

ETHICS AND DISSEMINATION

Ethics

The ~~trial-study~~ has full ethical approval from the National Research Ethics Service (NRES) Committee East Midlands (REC reference 11/EM/0410). It will be conducted according to the principles of the Medical Research Council Good Clinical Practice guidelines, Declaration of Helsinki, Data Protection Act, NHS Research Governance and relevant local and national laws. All patients will ~~give-provide~~ written informed consent during their first visit, before any tests are carried out, and will have had at least 24 hours to decide whether to participate or not prior to this.

Study Organisation and Oversight

The Sponsor is the University Hospitals of Leicester NHS Trust. A Trial Steering Committee (TSC) has been appointed and is responsible for the scientific and ethical conduct of the study. This consists of the an Independent chairman and two members, the Chief Investigator, two co-investigators from the tertiary centres, a lay representative, representative of the Clinical Trials and Evaluation Unit and a

representative of the Sponsor. The ~~trial-study~~ protocol and subsequent amendments have been approved by the TSC. A data monitoring committee was deemed not necessary given the observational design.

Study Timetable

Ethics application was approved in December 2011. Study enrolment started in April 2012 and recruitment is expected to be completed in November 2013 with a further 12 months for follow-up, post-processing and close-out of the study. The main study paper will be submitted within six months of study close-out.

FUNDING SOURCES

The study has been funded by a grant from the National Institute of Health Research, (NIHR) (Grant award number: NIHR-PDF 2011-04-51 Gerald P McCann). Additional support and resources for the ~~trial-study~~ will be provided by the NIHR Leicester Cardiovascular Biomedical Research Unit and NIHR Comprehensive Local Research Networks.

DISCUSSION

Limitations

The primary endpoint includes AVR for symptom development, which is subjective in nature. However, AVR by itself is deliberately not considered an endpoint due to the variability in clinical practice in referring severe but asymptomatic patients for surgery. Such patients will also be excluded from primary endpoint analysis. The

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6 presence of underlying coronary artery disease (CAD) may affect MPR and possibly
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8 symptom development. Although previous CABG / MI with 6 months is an exclusion
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10 criteria, the patients will not undergo coronary angiography to exclude CAD. The
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12 possibility of CT coronary angiography was considered but the high radiation dose at
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14 the time of study planning, administration of intravenous contrast and beta-blockers,
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16 was not felt to be justified in asymptomatic patients.
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20 **Anticipated Health Benefits**

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23 The study will address a number of limitations in previously published data, with the
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25 primary endpoint being driven by symptom development and MACE. The study
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27 should identify the strongest prognostic markers on which to base identification of
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29 asymptomatic patients with moderate to severe AS for consideration of early (before
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31 symptoms develop) AVR. The efficacy of such a strategy should be assessed in a
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33 prospective randomised controlled trial of early surgery in those with impaired MPR
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35 versus watchful waiting until symptom development.
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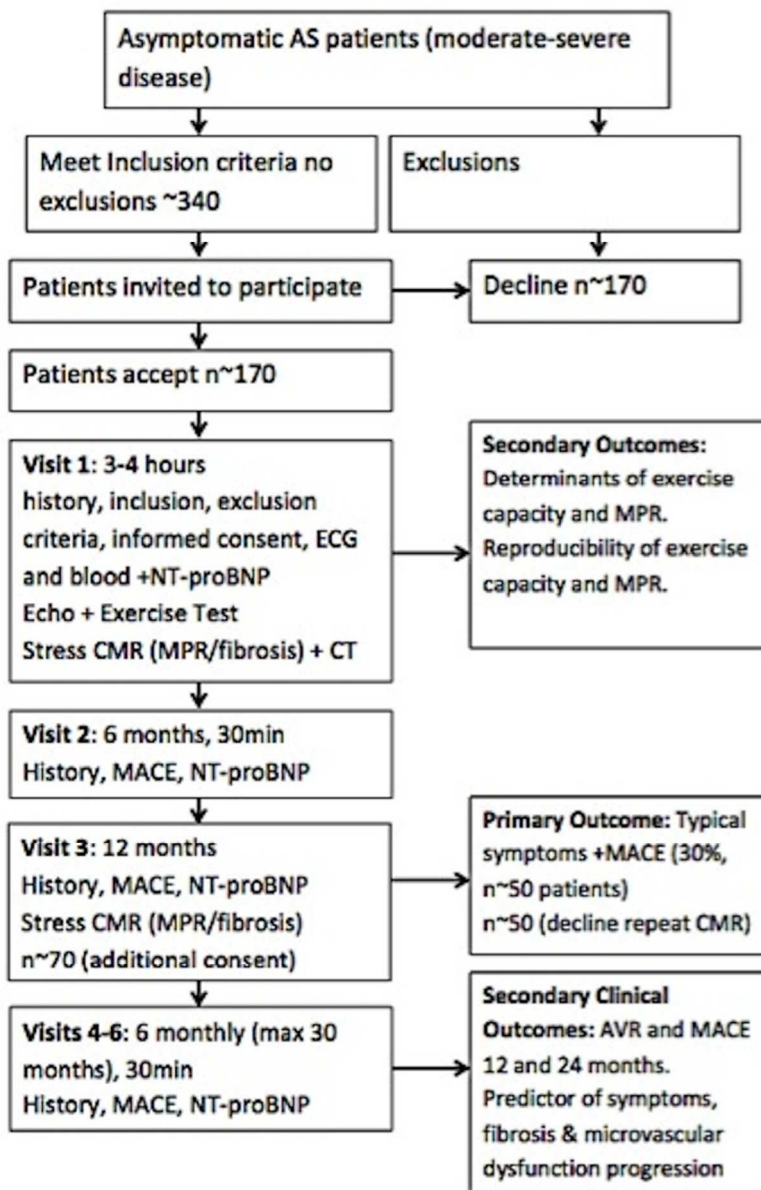
FIGURE LEGENDS

Figure 1: Flowchart demonstrating study plan

Figure 2: MRI Protocol used (Abbreviations- 4/2/3C: 4/2/3 chamber, LV: left ventricular, LA: left atrial, LVOT: left ventricular outflow tract; LGE: late gadolinium enhancement)

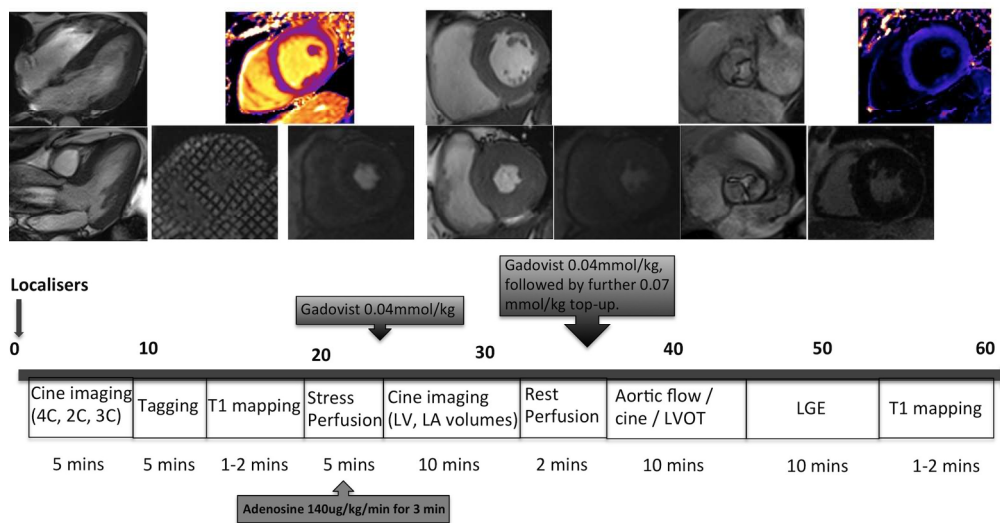
AUTHORS' CONTRIBUTIONS

AS is responsible for acquisition, analysis and interpretation of data and drafted the manuscript. IF and MJH are responsible for analysis and interpretation of data and critical revision of the article for important intellectual content. JPG, CB, SN, BP, BW and NJS were involved in conception and design of the study and revising the manuscript critically for important intellectual content. JNK and AU were involved in acquisition of data and revising the manuscript critically for important intellectual content. GPM is responsible for the conception and design of the study, drafting and critically revising the manuscript for intellectual content. All authors gave final approval of the manuscript.



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