Understanding the Pathophysiology of Aortic Valve Dysfunction using Advanced CMR Techniques: 4D Flow in Aortic Valve Disease

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I DEDICATE THIS WORK TO MY FATHER

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DECLARATIONS

The work in thesis is my own and is original. Dr. Victoria Stoll provided healthy volunteer whole heart 4D flow data for Chapter 6. Joanne Sellwood assisted with recruitment of patients. Dr. Sean Coffey assisted with and performed much of the statistical analysis of the OxVALVE data, in conjunction with the statistics support team at the Biomedical Research Council Oxford. Dr. Aaron Hess provided technical support and coding for the 4D flow sequences.

ABBREVIATIONS

2D	Two-dimensional
3D	Three-dimensional
4D flow	Three dimensional, three directional, time resolved velocity encoded magnetic resonance imaging
AA	Ascending aorta
ACEi	Angiotensin converting enzyme inhibitor
AF	Atrial fibrillation
ANOVA	Analysis of variance
ARB	Angiotensin receptor blocker
AS	Aortic stenosis
ASC	Aortic sclerosis
AVA	Aortic valve area
AVAi	Aortic valve area indexed to body surface area
AVC	Aortic valve calcification
BAV	Bicuspid aortic valve/bicuspid aortic valves
BMI	Body mass index
BP	Blood pressure
BSA	Body surface area
CABG	Coronary artery bypass grafting

CMR	Cardiovascular magnetic resonance
CMR-FT	Feature tracking cardiovascular magnetic resonance
CO	Cardiac output
COi	Cardiac output indexed to body surface area
COV	Coefficient of variance
CPET	Cardiopulmonary exercise testing
CT	Computed tomography
CVA	Cerebrovascular accident
EF	Ejection fraction
ECG	Electrocardiogram
ED	End diastole
EDSR	Early diastolic strain rate
EDV	End diastolic volume
ELI	Energy loss index
ES	End systole
GP	General practitioner
HASTE	Half-Fourier single-shot turbo spin-echo
HLA	Horizontal long axis
IVS	Interventricular septum
KE	Kinetic energy
LA	Left atrium

LV	Left ventricle
LVEDD	Left ventricular end diastolic dimension
LVESD	Left ventricular end systolic dimension
LVEDV	Left ventricular end diastolic volume
LVEDVi	Left ventricular end diastolic volume indexed to body surface area
LVESV	Left ventricular end systolic volume
LVESVi	Left ventricular end systolic volume indexed to body surface area
LVH	Left ventricular hypertrophy
LVOT	Left ventricular outflow tract
MAP	Mean arterial pressure
MR	Magnetic resonance
NS	Not significant
PC MRI	Phase contrast MRI
PDA	Proximal descending aorta
PWV	Pulse wave velocity
RER	Respiratory exchange ratio
SA	Short axis
SD	Standard deviation
SEM	Standard error of the mean
SNR	Signal to noise
SR	Strain rate

SSFP	Steady state free precession
STJ	Sinotubular junction
SV	Stroke volume
SVi	Stroke volume indexed to body surface area
SW	Stroke work
SWi	Stroke work index
T	Tesla
TAV	Tricuspid aortic valve/tricuspid aortic stenosis
TAVI	Transcatheter aortic valve replacement
TE	Echo time
TIA	Transient ischaemic attack
TKE	Turbulent kinetic energy
TR	Repetition time
VENC	Velocity encoding
VHD	Valvular heart disease
VO_2	Oxygen consumption
WSS	Wall shear stress
ZVA	Valvulo-arterial impedance

TABLE OF CONTENTS

ACKNOWLEDGEMENTS ..... **III**

DECLARATIONS ..... **V**

ABBREVIATIONS ..... **VI**

TABLE OF CONTENTS ..... **X**

LIST OF FIGURES ..... **XIV**

LIST OF TABLES ..... **XV**

ABSTRACT ..... **XVI**

1 INTRODUCTION AND AIMS **1**

1.1 Overview and General Background 1
1.2 The Development of Calcific Aortic Valve Disease 4
1.2.1 Normal Development and Flow 4
1.2.2 Bicuspid aortic valve development and flow 5
1.2.3 The development of aortic valve calcification 7
1.3 Afterload 10
1.3.1 Non-invasive assessment of central blood pressure and pulse waveform analysis 11
1.3.2 Aortic distensibility and stiffness 11
1.3.3 Kinetic energy conservation in the ascending aorta 13
1.3.4 The impact of afterload on the left ventricle 14
1.4 Novel Imaging tools in the Assessment of AVC 15
1.4.1 Overview of current imaging techniques 16
1.4.2 Cardiac Magnetic Resonance Techniques. 16
1.4.3 Novel CMR techniques of *in vivo* flow assessment 18
1.4.4 Physiological testing in AS 19
1.5 Aims and Hypotheses 20

2 GENERAL METHODOLOGY **23**

2.1 Subject recruitment 23
2.2 Definitions of aortic sclerosis & stenosis 25
2.3 Study Design 25
2.4 Study Protocol 26
2.4.1 Clinical assessment 26
2.4.2 Non Invasive Pulse Wave Analysis 26
2.4.3 Cardiac magnetic resonance imaging protocol/data acquisition 27

2.4.4 Left ventricular assessment 29
2.4.5 Left atrial assessment 31
2.4.6 Aortic valve assessment 31
2.4.7 Aorta 32
2.5 3 Tesla assessment of 4D flow 34
2.5.1 Assessment and visualisation of flow 34
2.5.2 Flow angle and displacement 38
2.5.3 Wall shear stress 38
2.5.4 Turbulent kinetic energy assessment 40
2.5.5 Whole heart 4D flow 41
2.5.6 Statistical analysis 44

3 AORTIC SCLEROSIS: CURRENT COMMUNITY PREVALENCE AND ASSOCIATIONS 46

3.1 Abstract 46
3.1.1 Background and Aims 46
3.1.2 Methods 46
3.1.3 Results 46
3.1.4 Conclusion 47
3.2 Introduction 48
3.3 Methods 50
3.4 Results 53
3.4.1 Main demographic findings (Unmatched data-Table 3-1) 53
3.4.2 Associations with existing cardiovascular disease (unmatched data-Table 3-1) 54
3.4.3 Echocardiographic findings in unmatched population (unmatched data-Table 3-1) 54
3.4.4 Comparison of matched data (Table 3-2) 56
3.4.5 Sensitivity analysis between those with hypertension with and without ASC (matched data Table 3-3) 58
3.4.6 Sensitivity analysis between those without hypertension with and without ASC (matched data Table 3-4) 58
3.5 Discussion 61
3.5.1 Main findings 61
3.5.2 Prevalence comparison with other studies 61
3.5.3 Associations and links with hypertension 64
3.5.4 Comparison of cohorts with and without hypertension and ASC 65
3.6 Limitations 67
3.7 Conclusions 67

4 AORTIC GEOMETRY, FUNCTION AND FLOW 69

4.1 Abstract 69
4.1.1 Background and Aims 69
4.1.2 Methods 69
4.1.3 Results 70
4.1.4 Conclusion 70
4.2 Introduction 72
4.3 Methods 73
4.4 Results 76
4.4.1 Participant characteristics 76
4.4.2 Afterload (2): aortic dimensions and turbulent kinetic energy 80
4.4.3 Correlations with turbulent kinetic energy 83

4.4.4 Break down of wall shear stress 84
4.5 85
4.5 Discussion 86
4.5.1 Summary of results 86
4.5.2 Advanced measures of blood pressure and aortic stiffness 86
4.5.3 Aortic dimensions and peak systolic turbulent kinetic energy 88
4.5.4 Flow angle and WSS 90
4.5.5 Visual inspection and grading of flow 91
4.5.6 Regional WSS 92
4.6 Limitations 93
4.7 Conclusions 94

5 LEFT VENTRICULAR RESPONSE: GEOMETRY, MASS AND FUNCTION 95

5.1 Abstract 95
5.1.1 Background and aims 95
5.1.2 Methods 95
5.1.3 Results 96
5.1.4 Conclusions 96
5.2 Introduction 97
5.3 Methods 99
5.4 Results 100
5.5 Discussion 106
5.5.1 Aortic Sclerosis 106
5.5.2 Aortic Stenosis 107
5.5.3 Limitations 111
5.6 Conclusions 111

6 CARDIOPULMONARY EXERCISE TESTING AND WHOLE HEART FLOW; A COMPARISON OF ESTABLISHED BICUSPID AND TRICUSPID AORTIC STENOSIS 112

6.1 Abstract 112
6.1.1 Introduction 112
6.1.2 Methods 112
6.1.3 Results 112
6.1.4 Conclusion 113
6.2 Introduction 114
6.3 Methods 118
6.3.1 Cardiopulmonary Exercise Test 119
6.3.2 Whole Heart 4D Flow 119
6.3.3 Analysis 120
6.4 Results 121
6.5 Discussion 125
6.6 Conclusion 127

7 GENERAL CONCLUSIONS AND FUTURE DIRECTIONS 128

APPENDIX: 1ST AUTHOR ABSTRACTS ARISING FROM THIS WORK AND RELATED PUBLICATIONS 132

7.1.1 Finalist of Young Investigator of the Year Award: 11th British Society of Cardiac Magnetic Resonance Annual Meeting 2016 132
7.1.2 Finalist of Young Investigator of the Year Award: British Heart Valve Society 2015 132
7.1.3 Oral Presentation: SCMR and EuroCMR Conference Nice 2015 132
7.1.4 Oral Presentation: Heart Valve Society of America, New York 2014 133
7.1.5 High Scoring Abstract and Moderated Poster, EuroEcho 2012 133
7.1.6 Moderated Poster, EuroCMR Vienna 2014 133
7.1.7 Selected Related Publications 133

8 REFERENCES 135

LIST OF FIGURES

Figure 1-1. Aortic leaflet composition and relationship to aorta.. 4
Figure 1-2. Normal flow patterns aiding closure of aortic valve.. 5
Figure 1-3. Flow patterns in bicuspid aortic valve disease..... 6
Figure 1-4. Evolution of aortic valve calcification 8
Figure 2-1. Non invasive pulse wave analysis profile. 27
Figure 2-2. 1.5T imaging protocol. 28
Figure 2-3. 3T imaging protocol. 36
Figure 2-4. Visualisation of cross sectional flow profiles at the mid-ascending aorta. 37
Figure 2-5. Schematic of Flow Angle.. 38
Figure 2-6. Wall shear stress. A schematic demonstrating the directions of shear stress in a vessel. 39
Figure 3-1. Prevalence of aortic sclerosis by age in years 54
Figure 4-1. Segmentation of the aorta. 75
Figure 4-2. Perpendicular assessment of flow angle at the level of the pulmonary artery. 75
Figure 4-3. WSS (magnitude) across 12 aortic segments 84
Figure 4-4. WSS (in plane) across 12 aortic segments..... 85
Figure 4-5. WSS (through plane) across 12 aortic segments..... 85
Figure 6-1. Left ventricular flow components. 115
Figure 6-2. Typical kinetic energy profile (in health) for the four flow components. 116

LIST OF TABLES

Table 2-1. General inclusion and exclusion criteria. 24
Table 2-2. Inclusion and exclusion criteria for CMR studies. 24
Table 2-3. Definitions of AS from the ESC. 25
Table 2-4. Intra and inter-observer variability of whole heart 4D flow. 44
Table 3-1. Main results from unmatched population. 55
Table 3-2. Matched Data for age, gender and cardiovascular disease. 57
Table 3-3. Matched data for hypertensive subjects..... 59
Table 3-4. Matched data for non-hypertensive subjects. 60
Table 3-5. Summary of major epidemiological studies of aortic sclerosis. 62
Table 4-1. Participant demographics. 76
Table 4-2. Measures of aortic stiffness. 78
Table 4-3. Correlations of distensibility at the level of the mid ascending aorta..... 79
Table 4-4. Adjusted analysis covariance for age between aortic sclerosis and controls. .. 80
Table 4-5. Aortic dimensions, flow and turbulent kinetic energy. 82
Table 4-6. Correlations with peak turbulent kinetic energy. 83
Table 5-1. Demographics, LV volumes, mass and function. 101
Table 5-2. Results of peak systolic strain and strain rate (SR)..... 102
Table 5-3. Correlations with mass index. 103
Table 5-4. Correlations with peak turbulent kinetic energy. 104
Table 5-5. Correlations with indexed aortic valve area 105
Table 6-1. Participant demographics, LV volumes, function and basic CPET outcome.... 122
Table 6-2. Whole heart 4D flow components and end-diastolic kinetic energy in AS. 123
Table 6-3. Demographics and 4D flow results of AS and control group. 124

ABSTRACT

Aortic valve disease is common with early valve thickening (known as aortic sclerosis, ASC) affecting around 30% of those over 65 years of age, and nearly 3% of the population over 75 years old having haemodynamically significant aortic stenosis. During clinical follow up of aortic stenosis, little distinction has been made between those with congenitally bicuspid aortic valve disease (2% of the population) (BAV AS) and those with acquired degenerative disease (TAV AS), although these contribute roughly equal proportions of aortic valve surgery.

I demonstrated that ASC is more common than previously thought with a prevalence of 38.5% in the over 65 year old general population and was strongly related with age, hypertension and a history of ischaemic heart disease (all $p<0.05$). In an age matched population without pre-existing hypertension but with ASC, I found those with ASC have a statistically significantly higher blood pressure than those without ASC (mean systolic blood pressure difference of 4.4mmHg; $p=0.004$). However, in a population with known hypertension and ASC, no prescribing differences in antihypertensive agents exist and no differences in the absolute level of hypertension were found.

In a CMR sub study, markers of aortic stiffness were more impaired in both ASC and established tricuspid AS disease to a similar degree, suggesting a fully expressed vascular phenotype exists before progression to valvular AS, when compared with control and BAV AS. However, there was a mean age difference of about 8 years between ASC and BAV AS

and control groups, which confounds the result. After age adjustment, a faster pulse wave velocity between ASC and controls remained (19.02 m/s compared with 14.4 m/s, p=0.028).

TAV and BAV AS have distinct differences in aortic dimensions and turbulent kinetic energy formation was greater in BAV AS, implying irreversible pressure loss ($p<0.0001$). Turbulent kinetic energy correlated to valve area ($r=0.53$, $p=0.001$) and very strongly to LV mass in a BAV AS population ($r=0.83$; $p=0.0001$), implying greater LV work may be required to compensate for the higher turbulent kinetic energy, particularly in the BAV group. The patterns of wall shear stress (WSS) on the aorta did not differ in intensity but did have differences in peak sites of the aortic wall in TAV and BAV AS. Impaired distensibility in the aorta was seen in both TAV and BAV AS, which appears to be more regional (confined to the ascending aorta) in BAV AS.

Turbulent kinetic energy emerged as a novel and interesting marker in AS, especially in BAV AS, showing moderate or strong correlation with LV mass, volumes, longitudinal strain and stroke work, and may go some way to explain the previously noted weaker than expected correlations between LV mass and valve area in AS. Future longitudinal studies to relate turbulent kinetic energy more precisely to LV function and outcome are warranted.

1 INTRODUCTION AND AIMS

1.1 OVERVIEW AND GENERAL BACKGROUND

In health the aortic valve is a trileaflet structure, which maintains unidirectional flow from the left ventricle into the aorta. Aortic valve calcification (AVC) refers to structural changes from early valve thickening termed aortic sclerosis (ASC), through to haemodynamically important calcific aortic valve narrowing, termed aortic stenosis (AS). Once thought to be passive degenerative change, AVC is now recognised to be a metabolically active process characterised by inflammatory, and latterly calcific, endothelial and valvular change.[1, 2] Several uncertainties surrounding the development and progression of AVC exist. ASC affects around 30% of those over the age of 65 years and has a distinct, adverse cardiovascular risk profile.[3] It is associated with excess cardiovascular morbidity and mortality with 1.5 times the number of adverse events occurring during 5 year follow up, compared with a population without ASC.[4] Of those with ASC, only about 5% develop clinically important AS over 7 year follow up.[5] Rather than simply representing a usually non-progressive precursor to AS, ASC may represent a distinct vascular phenotype, and better understanding of its nature as a marker of cardiovascular risk is needed.

In the minority in whom AS develops, it is almost always progressive and a critical point arises when symptoms develop and left ventricular (LV) decompensation occurs. If no intervention takes place, heart failure and death result.[6] This critical valve area varies considerably between even apparently similar individuals.[7] Some of the reasons for this

variability will be explored in this thesis but another basic distinction in AS applies; leaflet number.

A sub group of 1-2% of the population are born with only two functioning leaflets, so called bicuspid aortic valve (BAV) disease.[7] These valves are less mechanically robust than normal aortic valves and many go on to require valve surgery in their lifetime. Together with antecedent monitoring and long term post-operative monitoring, they form a significant burden to cardiovascular healthcare.[8, 9] Of those under the age of 70 years who undergo aortic valve replacement for aortic stenosis, 60% have a bicuspid valve (dropping to 40% of those over 70 years), meaning BAV is over-represented in subjects undergoing valve surgery.[10]

As demonstrated by the figures above, BAVs are less durable and patients tend to develop disease a mean of two decades earlier than those with acquired disease of a trileaflet aortic valve (TAV).[10, 11] While BAV AS is often followed up and treated in a similar way to TAV AS, there are important distinctions. BAV patients often develop a dilated ascending aorta with attendant risk of life-threatening dissection, rarely seen in TAV disease.[12-14] Studies of flow patterns have demonstrated abnormal flow in the majority of BAV patients with and without valve disease; this phenomenon is almost never seen in a healthy TAV.[15, 16] Small-scale studies suggest that LV function may be subtly abnormal even prior to the development of overt valve disease, leading to the hypotheses that BAV is associated with a cardiomyopathy or that flow jets into a dilated aorta create turbulent kinetic energy and thus pressure loss which increases LV work leading to a decline in function over time.[17, 18]

Despite the differences between BAV and TAV AS, little distinction is made concerning leaflet morphology in much of the available research data (including clinical trials). In clinical practice the two forms of AS are usually followed up at similar time intervals.

In this thesis, current prevalence data, associations with cardiovascular disease and relationships between hypertension and ASC are explored using large-scale population echocardiographic and demographic data. The association of ASC with basic LV echocardiographic parameters are described. Developing from this background, novel cardiac magnetic resonance sequences were used to describe detailed aortic flow and function, and to compare the vascular phenotype between ASC with established AS, and BAV and TAV AS. For the first time flow changes, aortic wall shear stress and the formation of turbulent kinetic energy in the proximal aorta were described in ASC and TAV AS. Detailed CMR sequences were then used to determine the associations of aortic afterload on the LV. Finally, LV flow and physiological output are compared between BAV and TAV AS, using cardiopulmonary exercise testing and new CMR sequences which describe the conservation of kinetic energy use in the LV.

1.2 THE DEVELOPMENT OF CALCIFIC AORTIC VALVE DISEASE

1.2.1 Normal Development and Flow

The normal aortic valve develops *in utero* around the 4th week of gestation, as part of the septation of the truncus arteriosus, ultimately giving rise to the pulmonary and arterial circulations. The aortic valve is semi-lunar and formed from fusion of the endocardial cushions. Abnormalities of the fusion process create a bicuspid valve.

The aortic valve leaflets are composed of three layers and run in continuum with the aorta itself as shown in Figure 1:1:

i. The fibrosa, providing strength and structure to the valve
ii. The spongiosa, which is elastin rich and acts as a buffer
iii. The ventricularis, which is rich in elastin and provides additional strength and stretch to the valve

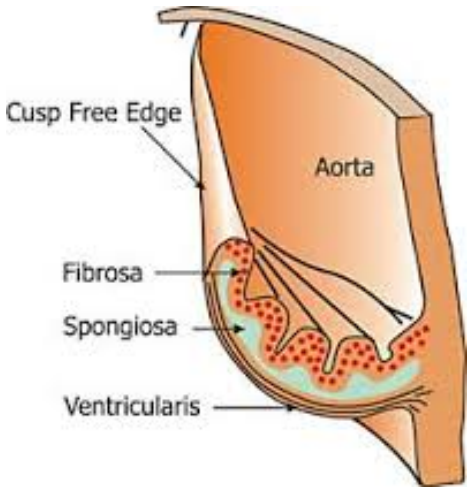


Figure 1-1. Aortic leaflet composition and relationship to aorta. The three layers are shown from the ventricular to aortic side; ventricularis, spongiosa, fibrosa.

Efficient valve function is dependent upon the flow patterns generated by the morphology of the sinuses of Valsalva and sinotubular junction. Flow studies have shown that leaflets open with forward flow, while vortical flow within the sinuses starts leaflet closure from end systole and optimise diastolic coronary artery flow.[19] The following diagram (Figure 1-2) illustrates the energy efficient action in health.

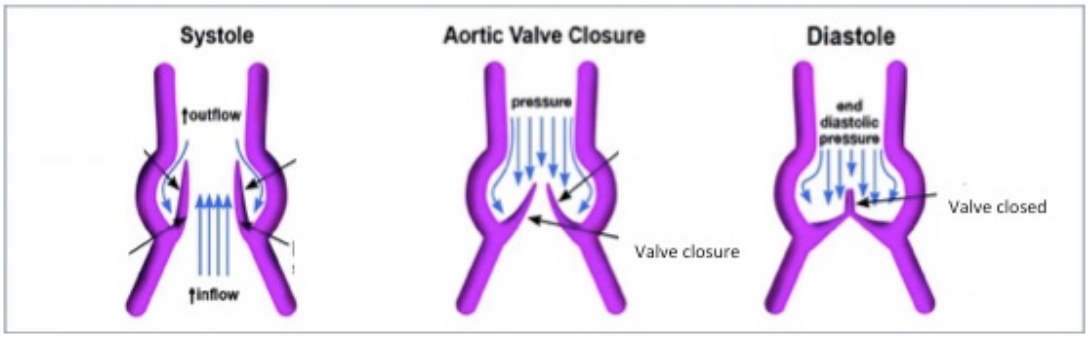


Figure 1-2. Normal flow patterns aiding closure of aortic valve. Forward flow opens the leaflets in systole with closure starting late systole with diastolic valve closure. Adapted from Otto Circ 2011[19].

In health, flow beyond the sinotubular junction and into the ascending aorta is laminar, or has a slight right handed helix.[15, 20] These optimal flow patterns create very little turbulence and kinetic energy is therefore well preserved in the normal aortic valve and root. However in BAV, normal flow patterns may be distorted, even with an apparently healthy valve, as described in the following section.

1.2.2 Bicuspid aortic valve development and flow

Most BAV arise from failure of right and left coronary cusp separation (74%).[21] The remainder are caused by failure of separation of the right and non-coronary cusp (16%).[21] Other morphologies are rare and are either left to non-cusp fusion or true bicuspid valves (in which no fusion line is seen).[22] Bicuspid valve disease is strongly, but not universally,

associated with aortic dilatation. Recent work speculates that abnormal flow patterns created in the valve-aortic continuum are implicated.[15, 20] Even BAV with near normal function have been shown to have a high prevalence of abnormal flow in the ascending aorta.[15, 16, 20]

Abnormal flow and with it, abnormal stress on the aortic wall termed wall shear stress (WSS), may partly explain the relationship between BAV and dilatation of the ascending aorta, but the extent of this role in propagating or perpetuating the process of dilatation is not fully understood.[15, 20, 23-25] Architectural abnormalities of the aortic wall are well described, but the extent to which these are primary or secondary to flow changes is unclear.[23, 26] If a BAV is diseased, these abnormal flow patterns are seen very commonly - usually exaggerated right handed helical flow, although disordered or left handed flow have also been observed.[15] The latter two flow patterns appear to be associated with more severe dilatation of the ascending aorta.[15, 25]

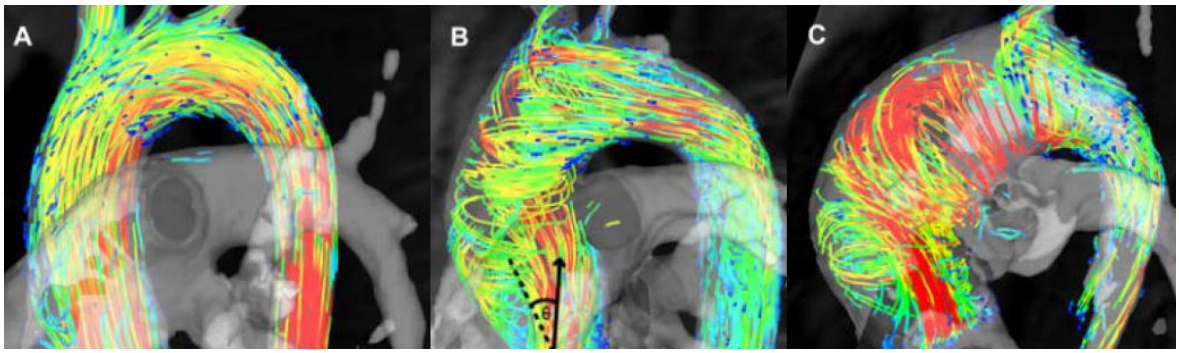


Figure 1-3. Flow patterns in bicuspid aortic valve disease; (A) Normal flow pattern; (B) Right-handed helical flow; (C) Left-handed helical flow. Adapted from Bissell 2013.[15]

Importantly, BAV is a congenital rather than acquired defect and the longstanding nature of flow changes may have implications for LV function due to alterations in afterload. Small studies have shown that patients with BAV, even those with normal valve opening,

show abnormal LV strain.[17, 18] Because of the findings of strain abnormalities, it has been suggested that BAV disease may also be a disease of the LV. It can be counter argued that the LV-aortic valve-aorta continuum is important and that flow or functional abnormalities of the aorta will cascade down to affect the LV, increasing afterload and LV work. This concept will be explored in Chapters 4 and 5 of this thesis.

1.2.3 The development of aortic valve calcification

1.2.3.1 Aortic Sclerosis

The common initiator in the development of both ASC and AS is endothelial damage provoked by repetitive forces on the valve leaflets, leading to endothelial breach and activation of the inflammatory cascade. This process is more likely with age, especially when accompanied by hypertension, which increases haemodynamic forces on the valve and with it the possibility of micro-trauma and subsequent inflammation.[19] The pathogenesis is very similar to the development of coronary artery plaques, with infiltration of the plaque by lipids triggering oxidised lipoprotein activity, stimulating further inflammation, subsequent fibrosis and calcific deposition (Fig1-4).[19]

Histological studies of early aortic valve calcification (AVC) demonstrate:[1]

- Inflammation with macrophage and T lymphocyte infiltration
- Focal accumulations of apolipoproteins (a), B and E
- ± Early calcification with increased expression of osteopontin

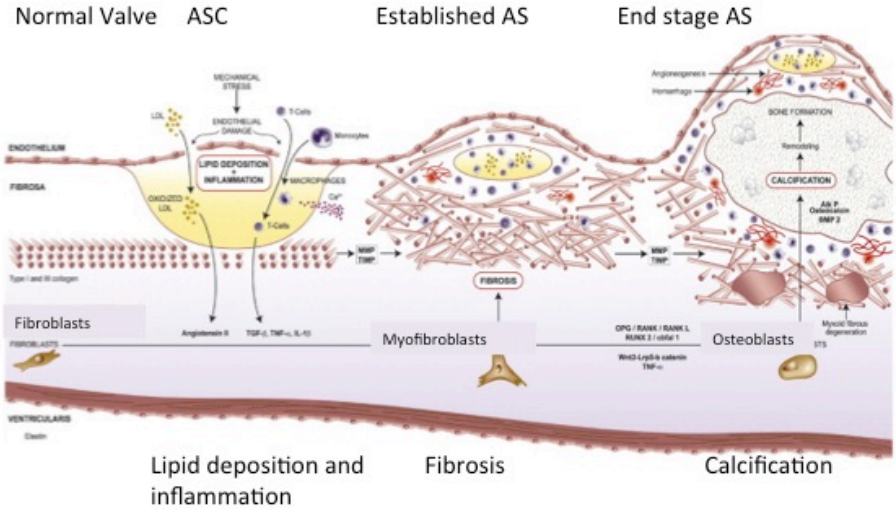


Figure 1-4. Evolution of aortic valve calcification (adapted from Dweck 2012)[1]. Normal valvular endothelium is on the left with progression from early leaflet thickening and inflammation to progressive fibrosis and calcification

Recent work has suggested that AVC involves two processes which are biphasic and more distinct than previously recognised.[1] ASC is primarily characterised by an intense inflammatory response on the valve leaflets with little or no calcification, whereas established AS is characterised much less by inflammation and more by abnormal calcification (even disordered bone formation) and little lipid.[1, 27, 28] While ASC and AS seem to share a common initiation, an unresolved trigger appears to cause a small proportion of subjects with ASC to develop haemodynamically significant AS.

1.2.3.2 Aortic Stenosis

Progression to AS is defined as leaflet thickening which creates enough restriction for valve opening to be considered haemodynamically relevant. In response to the original inflammatory process, fibrotic tissue remodels the extracellular matrix of valve tissue and fibroblast cells accumulate in the fibrosa layer. A proportion of these fibroblasts

differentiate into myofibroblasts, which in turn accelerate the fibrotic process. Matrix metalloproteinases are secreted and tissue angiotensin converting enzyme and angiotensin II receptors are up regulated. In late stage disease, myofibroblasts may differentiate to osteoblasts and recognisable local metabolic changes normally associated with new bone formation are found. In keeping with a predominantly calcific process, pathological specimens of severe AS demonstrate osteoblast and osteopontin expression with disordered laminar bone growth on the leaflets in severe cases.[28-30] The calcification starts deep in the valvular tissues at the hinge points and spreads inwards. Figure 1-4 summarises the process.

Exciting developments in metabolic imaging using ^{18}F-Sodium Fluoride tracer positron emission tomography scans (as a marker of active calcification) to display increased activity in the region of the valve as AS worsens have confirmed histological findings and demonstrate that AS is metabolically highly active.[27] For example, 97% of those with moderate disease and 100% of patients with severe disease displayed increased ^{18}F-Sodium Fluoride.[1]

The development of BAV AS appears to share the same pathophysiology as TAV AS but occurs at an earlier stage.[31] The bicuspid orifice is mechanically unfavourable and ineffective at distributing mechanical stress with unstable shear forces on the leaflets.[32] The ensuing micro-trauma initiates the inflammatory/calcific cascade leading to early valve degradation.[10, 11, 33]

Valvular stenosis creates a pressure load that the LV must overcome and forms a large part of the total afterload that the LV must overcome.

1.3 AFTERLOAD

A significant portion of the variability in symptom onset is the additional effects of post-valvular afterload on the LV, in the absence of a clear cause of LV dysfunction, such as past myocardial infarction or a cardiomyopathy.[34] The severity of remodelling and associated LV hypertrophy are governed by a number of factors including hypertension, age, genetics and gender.[35] The pattern of remodelling also varies and is recognised as concentric or asymmetric remodelling, whereby the wall thickness increases and cavity size decreases (i.e. LVEDV is lower) and concentric or asymmetric hypertrophy, whereby the wall thickness and cavity size increase (increasing both LV mass and LVEDV). [36]

Women tend to develop concentric hypertrophy, typically with a small, thick walled, LV cavity, while males tend develop eccentric hypertrophy, thick walls and a large cavity.[36, 37]

Genetic and epigenetic factors are increasingly understood but have complex interaction with environmental factors to produce adaptive and maladaptive myocardial changes.[38] Multiple candidate genes and pathways are being identified; including the role of calcineurin, G protein signalling and protein kinase C and sub families of mitogen-activated protein kinase in the degree and pattern of LVH.[39-44]

A major mechanical component of the afterload arises from i) overcoming arterial stiffness and ii) the “loss” of potential energy from the aorta as heat from turbulent kinetic energy.

1.3.1 Non-invasive assessment of central blood pressure and pulse waveform analysis

Aortic stiffness, however measured, is associated with increased mortality and cardiovascular morbidity, thought to be secondary to changes in ventricular vascular coupling and increased myocardial work in a population without valve disease.[45] Increased mechanical strain on the aorta causes fragmentation of elastin fibres and increase in collagen. Therefore, aortic wall stiffness and pulse wave velocity are increased, causing premature arrival of reflected pressure waves in late systole rather than diastole, increasing central pulse pressure and systolic blood pressure.[46, 47] This, in turn, increases LV afterload and myocardial work, ultimately resulting in concentric LVH, increased oxygen demand and potential sub-endocardial ischaemia.[48]

Standard sphygmomanometer measures of brachial blood pressure is a good measure of cardiovascular risk and end organ damage at large population levels.[49, 50] However, brachial blood pressure, while widely used, is less sensitive for the prediction of cardiac events and with the advent of non-invasive devices that measure central blood pressure there are increasing calls to use central pressure, at least in trials if not yet in clinical practice.[51-54] Devices such as Vicorder™ use pulse volume plethysmography to derive central and peripheral waveforms and have been validated for clinical use.[55]

1.3.2 Aortic distensibility and stiffness

The important contribution of vascular stiffness to the assessment of AS is now appreciated, with calls from the echocardiographic community to take account of global LV load, i.e. an estimation of the total valvular and vascular load (Zva), calculated as:[56]

$$Zva = (systolic\ arterial\ pressure + mean\ pressure\ gradient\ through\ valve) / stroke\ volume\ index$$

The effects of increased vascular resistance have been shown in outcome studies, comparing the development of symptoms in AS with and without hypertension, and demonstrating earlier adverse cardiovascular outcomes and LV remodelling in those with dual pathology.[57 58-60]

Local or regional aortic stiffness and well defined assessment of afterload is measured more directly as 'aortic distensibility' which has been shown to correlate with adverse cardiovascular outcomes in the general population[61, 62] and to relate to sub clinical LV dysfunction and functional status in AS.[63, 64] Aortic distensibility measures the relative change in cross sectional area, corrected for blood pressure. Closely related is 'pulse wave velocity', a measure of transit time of the aortic pressure wave down a specific length of the arterial tree. Faster times equate to stiffer vessels. Both are validated as markers of poor outcome in large studies.[65, 66]

This additional contribution of post valvular load is termed the 'double-load' on the LV. This often neglected additional load may go some way to explaining the commonly observed clinical scenario that individual patients with AS develop symptoms and LV decompensation at very different time points.[60]

ASC is strongly associated with hypertension, and aortic stiffness is likely to be markedly impaired in this group. BAV AS often have abnormal aortas and some work suggests this leads to impaired distensibility, even though they are younger on average than TAV AS.[67,

[68] Aortic stiffness, flow and function will be explored in Chapter 4 of this thesis and related to LV mass and function in Chapter 5.

1.3.3 Kinetic energy conservation in the ascending aorta

In AS, potential energy is lost from the ventricular valve aortic continuum as the jet accelerates through the valve, but a proportion of this is converted back to static energy. In patients with small aortas, the energy loss of decelerating blood converting kinetic energy back to static energy is less than in a dilated aorta, so there is more efficient energy conservation and reduced cardiac work. Therefore, the extent of pressure recovery is largely dependent on the ratio of the valve orifice area to the cross sectional area of the aorta.

The echocardiographic community have taken a lead with the development of the energy loss index (ELI) (aortic valve area index adjusted for pressure recovery), first postulated in 2000 but recently published with outcome data in a prospective study (a SEAS sub study of 1563 patients with asymptomatic AS).[69-71]

It is calculated as:

$$Energy\ loss\ index = \left[\frac{AVA\ x\ AA\ at\ STJ}{AA\ at\ STJ - AVA}\right]/BSA$$

Using this, the authors demonstrated that ELI has incremental independent prognostic value exceeding standard echocardiographic calculations of severity, and that a lower ELI was predictive of higher mortality over a period of 4.3 years follow up.[71]

ELI acts as a surrogate for the irreversible pressure loss that occurs when kinetic energy is converted to turbulent energy, which is in turn converted to heat and 'lost' from the system. Recently, it has become possible to measure this more directly, without assumptions and the inherent inaccuracy of echocardiographic measures. Early results from CMR studies have shown that measurement of turbulent kinetic energy in AS is feasible and correlates well with catheter based and echocardiographic calculations of pressure loss.[72]

The degree of turbulent kinetic energy formed is dependent on aortic size. This may be especially relevant in the BAV spectrum, due to aortic dilation. This dilatation may result, at least in part, from flow changes and marked deviation from normal laminar/slightly right handed helical flow has been seen in patients with BAV disease.[15] The full clinical significance of these flow abnormalities is not understood but it is likely that they represent a less efficient form of flow and that the larger aorta increases pressure loss. However, no studies on aortic flow in TAV AS have been published as a comparator and this work is presented in Chapter 4 of this thesis.

1.3.4 The impact of afterload on the left ventricle

Increasing afterload raises intra cavity pressure and the LV hypertrophies occurs as a mechanism to normalise wall stress.[73] While initially compensatory, LV hypertrophy is a marker of adverse outcome.[74-76] There are many complex interactions which govern the degree of hypertrophy but it is worse with concomitant hypertension, metabolic syndrome, diabetes, and in women.[77, 78] The hypertrophy associated with AS and hypertension is usually concentric but may also be eccentric or asymmetric.[36]

LV hypertrophy that occurs due to the hypertrophy of existing myocytes, is accompanied by an increase in collagen to provide structural support. However, these changes become maladaptive and fibrosis, which is only reversible in its early stages, develops. It is this diffuse fibrotic process that has recently been shown to be key in determining the outcome in AS.[79, 80] Once the fibrosis is irreversible, adverse changes occur in myocardial deformation and function.[79] Additionally, fibrosis can act as scar tissue, triggering arrhythmia.[79]

LV thickening and mass increase relate poorly to valve area alone, confirming the important role of post-valvular afterload as well as genetic and epigenetic factors. [35, 36] However, a major concern is that standard transthoracic echocardiographic parameters, the most widely used tool in AS monitoring, cannot directly detect adverse myocardial changes and that a drop in ejection fraction is a late and worrying development. Myocardial deformation using strain imaging is more sensitive to early LV decompensation and can be measured using echocardiography and CMR modalities.[36]

1.4 NOVEL IMAGING TOOLS IN THE ASSESSMENT OF AVC

The application of novel imaging techniques to AVC may provide novel insights into aortic function and flow and the effects on LV function. They may help elucidate some of the reasons for the variable responses seen to AS of apparently similar severity and may identify imaging biomarkers that merit longitudinal study.

1.4.1 Overview of current imaging techniques

Transthoracic echocardiography is the most widely used modality for the diagnosis, assessment and monitoring of AS. It has many advantages; it is cheap, non-invasive and widely available. The temporal resolution is excellent and assessment of velocity through the valve is accurate and reproducible if there is good alignment with the valve. However, it is reliant on good echocardiographic 'windows' for visualisation of the valve and myocardium. Even with excellent 'windows', views are limited to certain planes and orientations. The standard echocardiographic examination also relies on several assumptions; particularly the calculation of valve area using the continuity equation which assumes that the LV outflow tract is spherical and that the diameter has been measured correctly. Computed tomography (CT) scanning can be used to visualise the valve and calcification on the valve leaflets. It also offers excellent high-resolution visualisation of the aorta and coronary arteries, including accurate assessment of plaque burden and consequent stenoses. Simple LV function can be assessed but requires a relatively high radiation burden. Nevertheless, short prospectively gated low dose scans can give useful information about the valve area and coronary circulation including disease within the coronary vasculature.

1.4.2 Cardiac Magnetic Resonance Techniques.

CMR offers cross sectional imaging with no radiation burden. It has been validated for use in AS, with studies showing excellent agreement with valve areas measured by transoesophageal echocardiography.[81] CMR is the gold standard for the assessment of myocardial volumes, mass and function with excellent reproducibility and intra observer variability.[82, 83] Additionally, CMR can provide information about myocardial deformation

and tissue characterisation. It provides accurate functional and morphological data not only of the valve and LV, but also of the aorta and its properties. CMR assessment of the aorta allows visualisation of the whole vessel and planes can be selected and planned as desired to give cross sectional data, including maximum and minimum areas and derived distensibility.[84-86]

Ejection Fraction gives a relatively crude assessment of global LV function, a drop off in EF is a late and concerning finding in AS.[87] Cardiac strain imaging gives information about myocardial deformation, measured in the longitudinal, circumferential and radial planes. It has a number of advantages over ejection fraction, and is a marker of early LV decline.[88] Longitudinal strain in particular has been shown to equate with increasing myocardial fibrosis and a poorer outcome in echocardiographic studies of AS.[76, 88, 89] Evidence regarding circumferential strain in AS is mixed, with some authors suggesting it is supra-normal until a late stage, while others have found a drop.[89-91] It is a dimensionless index, defined as the change in myocardial fibre length at end systole compared with end diastole and expressed as a percentage:

$$Strain = (L - L0)/L0$$

where L= end systolic length and L0 is the end diastolic length.

Strain rate is strain expressed per unit time.

During LV contraction, strain values are negative; conversely in relaxation (diastole) strain is positive. The direction of strain is thought to partially reflect the underlying myocardial architecture with longitudinal, circumferential and radial strain reflecting endocardial, mid wall, and sub-epicardial function, respectively.

CMR strain images were analysed using 'feature tracking' software, which identifies features within a tissue voxel and tracks these through the cardiac cycle (repeated across multiple voxels). This allows cardiac motion and deformation to be assessed in a manner analogous to echocardiographic speckle tracking. Prior to this development, deformation could only be assessed by CMR using tissue tagging, a technique that was limited by long breath holds and fading of tag lines in diastole. Recent work has shown that in patients with AS, feature tracking analysis produces consistently higher values of longitudinal and circumferential global strain than does tagging.[92] Reproducibility was good for both global circumferential strain and longitudinal strain with poorer reproducibility for regional measures, notably apical circumferential strain. The authors suggest that tagging and feature tracking values cannot be used interchangeably. There may be better agreement at 3T, compared with 1.5T.[92] Studies by the same group, published in the same year but this time in myocardial infarction (1.5T), found much better inter-technique agreement between tagging and feature tracking and excellent inter and intra-observer variability.[93] The reasons for this variability are not clear, but may reflect disease specific variations.

1.4.3 Novel CMR techniques of *in vivo* flow assessment

Computational fluid dynamic studies have rapidly advanced alongside the development of advanced CMR techniques and can now quantify blood flow in 3 dimensions throughout the cardiac cycle.[94] This expansion of technology allows accurate *in vivo* visualisation of flow patterns within the heart and great vessels. Three-dimensional anatomical data are acquired simultaneously, giving the sequence the title of 4D flow (perhaps more correctly described as time-resolved 3D flow - 3D + time).

The data sets are processed and visualised in 3D format to give an impression of flow patterns. From these data, aortic parameters can be derived, including flow angle, flow rate/velocity and volume at any given point, helicity of flow and wall shear stress. Even newer sequences estimate turbulent kinetic formation and viscous energy loss.[72, 95] In the heart, patterns of blood flow can be measured and LV kinetic energy consumption broken down into 4 components of intra cardiac flow; direct flow (enters and leaves the LV in one cardiac cycle), delayed ejection flow (blood already within the LV which is ejected in the measured cycle), retained flow (which enters but does not leave the ventricle) and residual volume (already within the LV and does not leave).[96]

BAV flow patterns in the proximal ascending aorta differ with leaflet fusion pattern.[15] On going work will investigate the impact of flow abnormalities and the resulting increase in shear stress (predominantly outward) friction force on the aortic wall and its possible contribution to aortic dilatation.[20, 25] It remains unclear whether this is primarily a defect in the architecture of the aortic wall or whether the histological findings, including cystic medial necrosis and collagen replacement of elastin fibres, are a secondary response to abnormal flow and shear stress. No data from a population with TAV AS has been published to allow comparison of flow patterns, wall shear stress or kinetic energy, and these are presented in Chapter 4.

1.4.4 Physiological testing in AS

Standard treadmill or bicycle based exercise testing is recommended in guidelines for the assessment of asymptomatic AS, both to unmask symptomatic limitation and provide baseline data to guide future monitoring.[87, 97] As the demography of AS and treatment strategies have changed (older frailer individuals and the availability of minimally invasive

options), so standard exercise testing has become of more limited value. Factors that mitigate against successful exercise testing include arthritis, comorbidity and balance problems. Cardiopulmonary exercise testing (CPET) provides more information and allows the measurement of important parameters such as oxygen consumption and anaerobic threshold, as well as information about the cause of limitation (cardiovascular fitness, pulmonary disease or musculoskeletal fatigue).[98, 99] CPET could provide information concerning the physiological impact of valve morphology and is combined with whole heart 4D flow in a sub-study (chapter 6) of this thesis.

1.5 AIMS AND HYPOTHESES

The relationships between early (ASC) and late aortic valve calcification are incompletely understood. In established AS, there may be important differences in the LV response in BAV and TAV AS secondary to differences in the way energy may be lost (and retained) in the LV-valve-aortic continuum. CMR is ideally placed to explore these unknowns, using standard and novel sequences to explore flow and function within the aorta created as a consequence of valve disease and their impact on the LV. With improved understanding come options for treatment which have important health economic implications for protecting against excess cardiovascular risk in ASC, and may help to determine the optimal timing of follow up and valve (and root) replacement in AS.

The aims of this thesis are: (1) to describe the prevalence and cardiovascular risk relationship (especially hypertension) in ASC compared within the general population, (2) to describe flow patterns, aortic stiffness and the distribution of wall shear stress and the generation of turbulent kinetic energy in and on the aortas of patients with ASC, BAV and

TAV AS compared with a control group, (3) to determine the effects on LV function and relate them to the above factors, and (4) to establish the feasibility of whole heart 4D flow with physiological exercise testing in a sub group of patients with AS.

The following hypotheses are explored in this thesis:

- Hypertension is more prevalent in ASC and may be more difficult to treat in individuals with hypertension and ASC (compared with individuals with hypertension and no ASC) as a consequence of increased aortic stiffness. This was investigated in chapter 3, using data from the OxVALVE study and CMR techniques of aortic stiffness (distensibility, pulse wave velocity and assessment of pulse wave form using non-invasive pulse wave analysis assessments) in chapter 4.
- ASC is not haemodynamically unimportant. Early valvular abnormalities generate more turbulent flow and irreversible pressure loss. Combined with an additional afterload from aortic stiffness, LV function (strain) is likely to be impaired in ASC compared with healthy controls. This was investigated in chapter 4 and 5, using CMR measures of turbulence and LV strain.
- Flow patterns and function in the ascending aorta differ between BAV and TAV AS. Turbulent kinetic energy is higher in BAV AS due to the abnormal valve orifice and larger aortic root with implications for LV remodelling and function. Turbulent kinetic energy was measured with novel CMR sequences and standard measures of LV geometry and feature tracking LV strain was studied in chapter 4.
- Differences in aortic and LV response between BAV and TAV have physiological consequences, which are demonstrable with CMR and by CPET. The LV geometry and function was investigated in chapter 5 and CPET measures (peak VO_2 and)

together with data from whole heart 4D flow to examine flow and end diastolic kinetic energy was measured in chapter 6.

2 GENERAL METHODOLOGY

2.1 SUBJECT RECRUITMENT

Participants with ASC were identified and recruited from the on-going OxVALVE population cohort study (OxVALVE PCS), which uses transthoracic echocardiography as a screening tool to characterise the prevalence of valvular heart disease.[100] Other subjects with AS were recruited from clinics within the Oxford University Hospitals NHS Trust or studies within the Department of Cardiovascular Medicine, University of Oxford, where consent for contact had been provided. Favourable ethical opinion was obtained from the Oxfordshire regional ethics committee (12/SC/0599) with local approval from the Research and Development team at the Oxford University Hospitals NHS Trust (10298). All participants gave written informed consent.

Healthy controls were recruited from OxVALVE or prior imaging studies where any more than trivial or physiological valve disease had been excluded. All participants had to be willing to participate in a research study on a voluntary basis. Subjects were eligible if they were over 18 years old but were selected where possible to be 50-80 years old for improved group matching. The diagnosis of AS, ASC or no valve disease was based on previously documented echocardiography or CMR data, as indicated in section 2.2.

Overall exclusion criteria were more than mild other valve disease or an LV ejection fraction of less than 50%. Subjects with any rhythm other than sinus rhythm or

contraindication to CMR scanning (pacemaker, unsafe metal implants, severe claustrophobia) were also excluded.

The following inclusion and exclusion criteria were used specifically for the OxVALVE PCS cohort described in chapter 3:

Inclusion	**Exclusion**
≥65 years	Terminal illness
No known VHD	Immobility
Able to provide informed written consent	Frailty (including significant dementia)

Table 2-1. General inclusion and exclusion criteria.

The following inclusion and exclusion criteria were used for the CMR based study:

Inclusion	**Exclusion**
≥18 years	LV EF < 50%
Able to provide informed written consent	Rhythm other than sinus rhythm
Not pregnant/breastfeeding	More than mild other valve disease
Known AS, ASC, no VHD on recent imaging	Documented myocardial infarction
	No contraindication to CMR*

**Claustrophobia, pacemaker or defibrillator, pacemaker wires, aneurysm coils, cochlear implant, metal implant without known safety profile at 3T, shrapnel injury.*

Table 2-2. Inclusion and exclusion criteria for CMR studies.

2.2 DEFINITIONS OF AORTIC SCLEROSIS & STENOSIS

ASC was defined by transthoracic echocardiography as evidence of calcification on the aortic valve leaflets, accompanied by leaflet thickening but with a velocity through the aortic valve of <2.5m/s.

AS was defined according the European Society of Cardiology guidelines as valve orifice narrowing, with a velocity through the valve of >2.5m/s.[101] The following breakdown of severity was used:

	AVA (cm^2)	AVAi (cm^2/m^2)	Velocity (m/s)
Mild	>1.5	>0.85	2.6-2.9
Moderate	1.0-1.5	0.60-0.85	3.0-4.0
Severe	<1.0	<0.6	>4.0

Table 2-3. Definitions of AS from the ESC. AVA= aortic valve orifice area in cm^2. AVAi= aortic indexed to body surface area, Velocity= velocity of jet through valve in m/s.

2.3 STUDY DESIGN

The CMR component of the thesis is an observational case control study with four groups:

i. Controls without valvular heart disease: based on previous echocardiographic or CMR imaging from the OxVALVE study or recent studies within the department
ii. Aortic sclerosis volunteers (ASC): those with aortic sclerosis (as defined above) recruited from the OxVALVE study
iii. Tricuspid aortic valve stenosis (TAV): recruited from Oxford University Hospitals NHS Trust, or recent departmental (or OxVALVE) studies

iv. Bicuspid aortic valve stenosis (BAV): recruited from Oxford University Hospitals NHS Trust, or recent departmental studies

2.4 STUDY PROTOCOL

2.4.1 Clinical assessment

A basic medical and medication history and physical assessment including anthropometric measures (height, weight, waist circumference) and a standard 12 lead ECG were undertaken in all subjects.

2.4.2 Non Invasive Pulse Wave Analysis

Central and peripheral blood pressure and pulse wave analysis were measured using Vicorder™ proprietary software, with photo plethysmography based flow assessments which have been validated in independent studies and compare well with widely accepted applanation tonometry assessment for the assessment of pulse wave analysis.[102, 103]

The pulse wave analysis assessment took place immediately after 1.5T CMR assessment of aortic distensibility (>15 minutes lying supine). The waveform was averaged over 5-10 beats. From these data, blood pressure, central arterial pressure and augmentation index (the enhancement of central aortic pressure by a reflected wave) were taken, as shown in Figure 2-1.

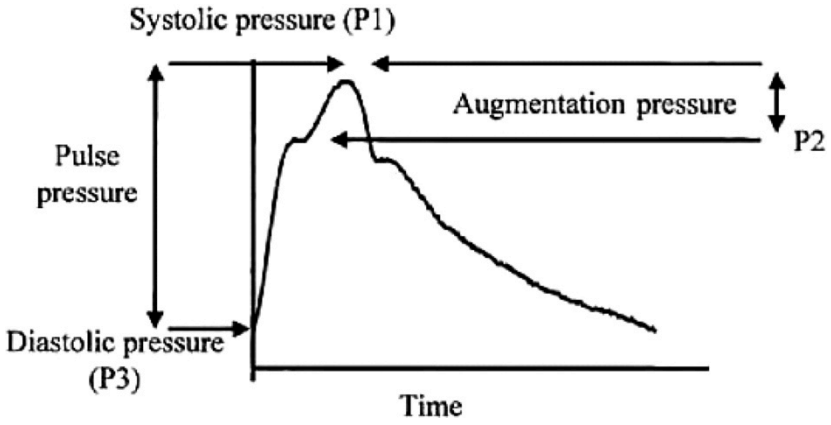


Figure 2-1. Non invasive pulse wave analysis profile. The pressure (y axis) is mapped against time (x axis) throughout a cardiac cycle

2.4.3 Cardiac magnetic resonance imaging protocol/data acquisition

1.5 Tesla scanning (Avanto, Seimens, Erlangen) using a 32 channel coil was performed to acquire standard anatomical and functional assessment of the LV, aorta and aortic valve, as well as high resolution assessment of aortic distensibility. After the 1.5T scanner, participants underwent 4D flow assessment at 3T (Trio, Seimens, Enlargen, Germany), which provides superior signal to noise ratio and improved data quality.[104]

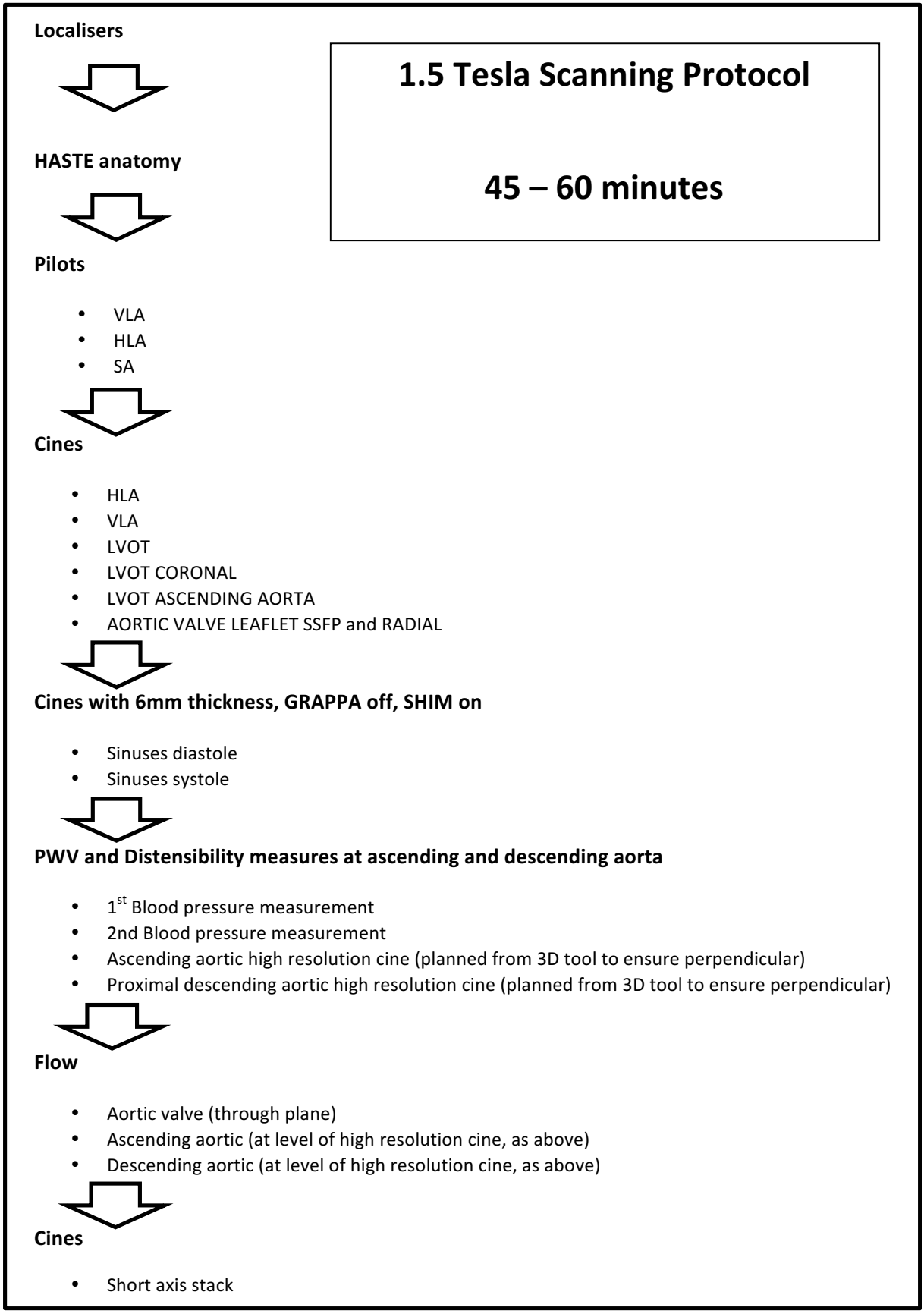


Figure 2-2. 1.5T imaging protocol.

2.4.3.1 1.5 T Imaging protocol

Localiser, half-Fourier single-shot turbo spin-echo (HASTE) anatomy, end expiratory cines of the LV, aortic valve and leaflet tips, and the aorta were acquired as outlined below. Standard phase contrast flow acquisition through the aortic valve, proximal and distal descending aorta (at the level of the bifurcation of the main pulmonary artery) was also acquired, as detailed below.

2.4.4 Left ventricular assessment

2.4.4.1 Volumes and mass

Steady state free precession (SSFP) imaging of the short axis stack was planned from horizontal and vertical long axis cine images. A slice thickness of 7mm with a 3mm gap was used. Imaging was at end expiration to minimise the effects of respiratory motion. Parameters were: TE 1.25 ms, TR 3.25ms, matrix 208 x 208, flip angle 60°, voxel 1.7 x 1.7 x 8mm. LV volumes and mass were calculated by manually contouring end systolic and diastolic epicardial and endocardial contours at each slice position, using CMR 42 software (Circle Cardiovascular Imaging, Calgary, Canada). LV ejection fraction (EF) and cardiac output (CO) were calculated:

$$EF = [SV/EDV]x100, CO = SV\ x\ heart\ rate$$

and indexed by body surface area. Myocardial mass was derived by subtracting the endocardial volume from the epicardial volume and calculated based on prior knowledge of myocardial specific gravity (1.05 g/cm^3).[105] The basal slice was defined as the first slice

to have >50% myocardium surrounding the lumen. Papillary muscles were excluded from analysis.

2.4.4.2 Function and strain

Feature tracking tracks uses standard SSFP cine images to track features of interest (dishomogeneities in tissue, anatomical landmarks and tissue interface) along contour lines.[106] Feature tracking software (Tom Tec, Unterchleissheim, Germany) was used to calculate peak global longitudinal and circumferential strain from SSFP cine images in the vertical long axis (VLA), horizontal long axis (HLA), and three short axis planes: basal (the first slice with a complete myocardial contour), mid LV (between the basal and apical slices) and the apical slice (the final slice with a complete myocardial contour).

The prescribed slices were analysed offline using a dedicated feature tracking platform (Tom Tec). The endocardial border was tracked across a cardiac cycle with manual correction of automated border detection, if required and propagated across the cardiac cycle. This produced endocardial strain values at given regions across the cardiac cycle. Peak systolic strain values were used. Global longitudinal strain was taken as the mean value of longitudinal strain obtained from the long axis views (VLA and HLA) and short axis strain was quoted for apex, mid and basal cavity values.

Published work using feature tracking in aortic stenosis has shown good intra and inter observer agreement for global circumferential and longitudinal strain.[92]

2.4.5 Left atrial assessment

LA area was calculated by taking the mean of the area in cm^2 traced at atrial diastole in the HLA and VLA views.[107]

The LA volume was calculated from the following formula:

$$(0.85x\ LA1x\ LA2)/mean\ maximal\ LA\ length$$

Where LA1 was the area in HLA, LA2 area in VLA and mean maximal atrial length taken from back wall to mitral valve hinge point in the long axis views.[108, 109]

2.4.6 Aortic valve assessment

2.4.6.1 Morphology and function

Aortic valve morphology (BAV or TAV) was assessed from thin-slice (4-5mm) radial SSFP sequences positioned across the valve leaflets, parallel to the short axis of the aorta, using the coronal and standard LVOT views for planning. These were positioned nearer the base of the valve than would be done for valve area to allow better visualisation of leaflet anatomy.

The aortic valve area was assessed using the same sequence and orientation, this time planned through the tips of the aortic valve as determined by close inspection of the movement of the leaflet tips through the cardiac cycle in LVOT and coronal LVOT images. A limitation of this technique can be that the true leaflet tips are missed - therefore the slice position was translated slightly up and down the planned views until the true orifice area had been captured. Using this technique, the CMR based planimetered area accords

well with those obtained using transoesophageal and transthoracic echocardiographic measures.[81]

2.4.6.2 Phase contrast flow

Phase contrast velocity mapping (standard 2D flow assessment) measures the velocity of the passage of blood by exploiting the phase shift of the MR signal in the moving blood, compared with the stationary tissue. Images were positioned across the narrowest part of the flow jet (the vena contracta) and acquired with through-plane velocity encoding. This technique can underestimate flow due to low temporal resolution in the face of fast moving jets and in states of high turbulence, and becomes increasingly inaccurate at velocities greater than about 4m/s.[110]

2.4.7 Aorta

2.4.7.1 Dimensions and geometry

A cine of the ascending aorta, arch and proximal descending aorta was acquired as a standard SSFP image using the HASTE images for positioning.

Aortic diameters were measured at end diastole from luminal edge to luminal edge, in accordance with American Heart Association guidelines.[111] Measurements were made perpendicular to the aortic lumen (planned to ensure a true short axis). Slices were taken at the sinotubular junction, and the ascending and descending aorta at the level of the pulmonary artery. All geometric dimensions were indexed to body surface area according to the Haycock formula.[112]

2.4.7.2 Distensibility

Aortic distensibility was measured in the ascending (AA) and proximal descending aorta (PDA) at the level of the pulmonary artery bifurcation. These were located at the same point as the cine images, i.e. perpendicular to the aortic lumen and acquired on expiratory breath hold using SSFP images, optimised in house to provide optimal temporal and spatial resolution.

Distensibility analysis was performed using a semi-automated Matlab tool using edge detection algorithms (The Mathworks Inc, Natick, MA, USA).[113] Distensibility was defined as the relative area change divided by the pulse pressure as below:

$$Compliance\ [mm2\ /\ mmHg\ ]\ =\ (max\ area\ -\ min\ area)\ /\ pulse\ pressure$$

$$Arterial\ strain\ [arbitrary\ units]\ =\ (max\ area\ -\ min\ area)\ /\ min\ area$$

$$Distensibility\ [1\ /\ mmHg]\ =\ arterial\ strain\ /\ pulse\ pressure$$

2.4.7.3 Pulse wave velocity

Pulse wave velocity (PWV) is a measure of the transit time of the aortic pulse wave and increases with increasing aortic stiffness. PWV was calculated by measuring the transit time from the proximal ascending to proximal descending aorta using the bifurcation of the pulmonary artery as a landmark. The length of the aortic centerline was measured manually on an oblique-sagittal slice through the aorta (HASTE scan sequence). The transit time was calculated between flow velocity curves using the half-maximum of the linear fit on the systolic upslope as the reference point on each curve. PWV was

calculated using in-house software based on active contour models with automatically segmented 2D phase contrast images.[114, 115]

2.5 3 TESLA ASSESSMENT OF 4D FLOW

2.5.1 Assessment and visualisation of flow

4D flow datasets were acquired on a 3 Tesla system (Trio, Siemens, Erlangen, Germany) using a 32-channel cardiac coil and retrospective ECG-gating. The flow-sensitive gradient-echo pulse sequence generates a 3-dimensional time-resolved magnitude image as well as 3 sets of directional flow images (in the x, y and z planes), which are subsequently combined to create a 3 dimensional flow dataset. The echo times were around 2.5ms with repetition times of around 5.1ms. The flip angle was 7°, the average temporal resolution 40ms and the voxel size 2.2 x 2.2 x 2.2mm^3. The velocity encoding value was individually determined for each patient based on the lowest value without aliasing, from a pilot scout scan. Diaphragmatic respiratory navigation was used to select slices to reduce breathing artefacts. (See Fig 2-3 for schematic of scanning protocol.)

The 3T protocol typically took 45-60 minutes to set up, plan and acquire the large data sets. The 4D flow acquisition sequence typically itself took 20-40 minutes. The variability was largely due to variation in breathing patterns, which affects the diaphragmatic navigation efficiency. Older patients seemed to have predominantly chest wall breathing, although this was an anecdotal observation. The retrospective sequence is only suitable for patients in sinus rhythm. Occasional ectopy did not affect the scan (other than

increasing the time) as ectopic cycles are excluded by the programming algorithm. However, very frequent ectopy can cause the scanner reconstruction limits to be exceeded and the scan to fail. Patients are counselled not to move as this causes mis-registration artefact, especially relevant in whole heart 4D flow.

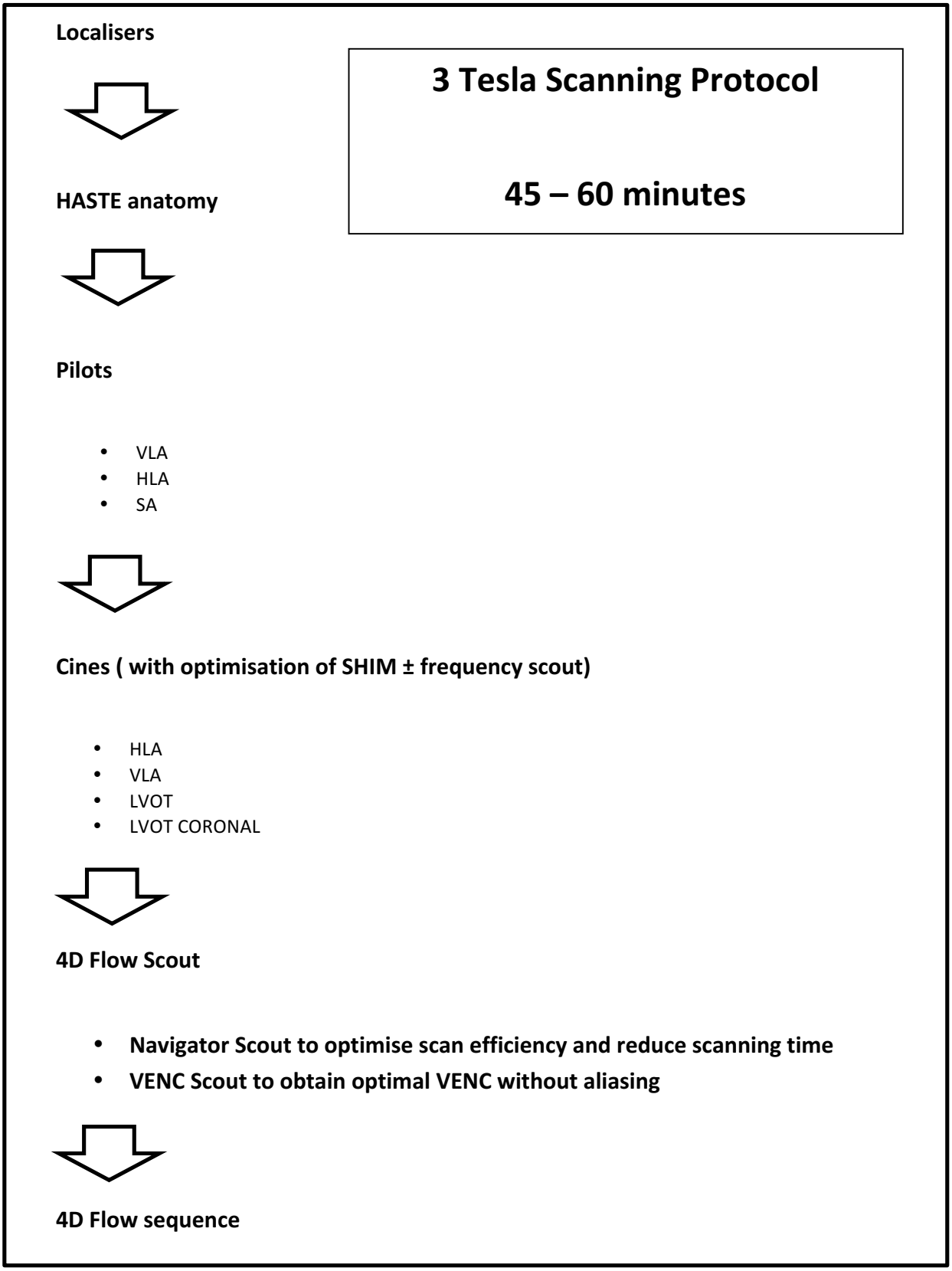


Figure 2-3. 3T imaging protocol.

Data processing and analysis used a combination of EnSight (CEI Inc, Apex, USA) Version 10.1.2 and Matlab plug-ins (The Mathworks, Inc, Natick, USA), which have been developed and distributed by several 4D, flow groups.[116] The datasets were corrected for eddy current and noise reduction, aliasing removal and static tissue subtraction. Pathlines indicating flow direction over time were used for visualisation and visual definition of helicity of flow.

Pathline visualisation is poor in older subjects and those with more severe AS due to drop out from a higher VENC encoding to avoid aliasing. It was not possible to produce long pathlines in some cases but flow eccentricity, derived as part of the wall shear stress analysis, provided a useful visual surrogate as shown below.

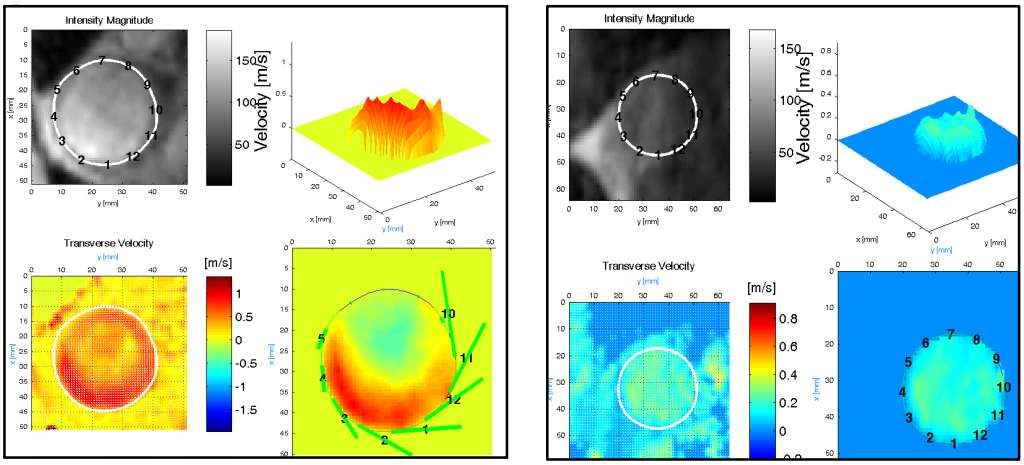


Figure 2-4. Visualisation of cross sectional flow profiles at the mid-ascending aorta. The left hand panel demonstrates eccentric flow in red against the aortic wall in a BAV AS. Lower more laminar flow in a control is in the right hand panel. Colour scales represent velocity of blood flow.

2.5.2 Flow angle and displacement

The degree of flow angle from a mid-aortic theoretical centre line was calculated as part of the wall shear stress assessment to define the degree of tangential flow (the flow angle), as shown below.[25, 117] Flow angle was measured from a short axis plane, perpendicular to the aortic walls, at the level of the bifurcation of the pulmonary arteries, as described by other authors.[15, 20, 25]

The systolic flow angle is the angle between the line perpendicular to the short axis analysis plane and the instantaneous mean flow vector at peak systole (Figure 2-5).[20]

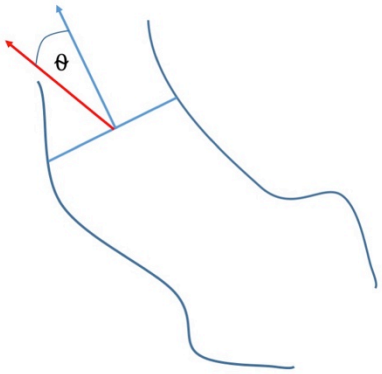


Figure 2-5. Schematic of Flow Angle. Flow angle is measured as the angle θ, between the line perpendicular to the short axis analysis plane (blue arrow) and the mean peak velocity direction (red arrow).

2.5.3 Wall shear stress

Wall shear stress describes the tangential force per unit area of blood as it flows against the endothelial surface of a blood vessel. It can be defined mathematically as:

$$WSS = \rho . \delta V / \delta r$$

Where ρ represents the viscosity of blood, V the velocity of blood, r the radius of the vessel, and δV/ δr the near-wall spatial velocity gradient at any instant.

WSS describes the shearing force of blood against the aortic luminal wall, which varies throughout the cardiac cycle. WSS in this thesis refers to systolic WSS. The degree of WSS varies by location and is described as through-plane (circumferential) and in-plane (axial) vectors. It can also be expressed as a single vector quantity of through plane and in plane (magnitude).

The analysis plane was placed at the level of the pulmonary artery and average values from one frame prior to and three frames after peak aortic systole were used (helical flow continues to develop after peak systole).

Markl *et al* assessed reproducibility, inter and intra observer variability for WSS. Inter observer variability gave a Pearson's correlation coefficient = 0.84 and Bland-Altman mean difference -0.02 with limits of agreement ± 0.09.[118] Intra observer variability for

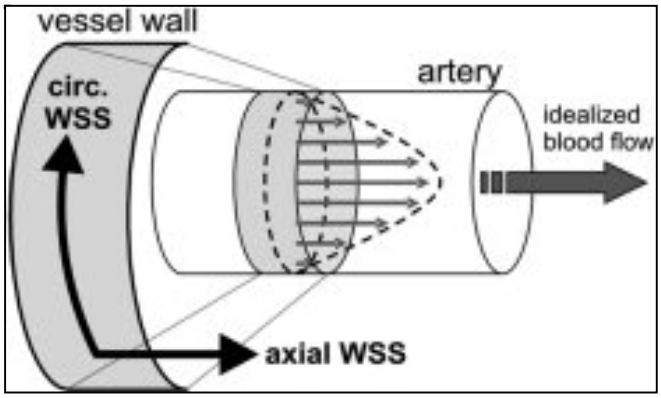


Figure 2-6. Wall shear stress. A schematic demonstrating the directions of shear stress in a vessel.

wall shear stress gave a Pearson's correlation coefficient = 0.87 and Bland-Altman mean difference of 0, with limits of agreement ± 0.07. The same group rescanned at one year, with a Pearson's correlation coefficient of 0.55 and Bland Altman mean difference of 0.03 with limits of agreement ± 0.14.[118] Within our own unit, intra observer variability on 5 control subjects gave a Pearson's correlation coefficient of 0.99 and Bland Altman mean difference of 0 with limits of agreement ± 0.02.

2.5.4 Turbulent kinetic energy assessment

Dyverfeldt *et al* developed a novel sequence to measure TKE, using existing 4D flow sequences. Signal amplitude between and within voxels is related to the distribution of velocities (lack of coherence, or chaotic flow and velocities are turbulent).

While phase contrast magnetic resonance (PC-MRI) mapping estimates mean velocities based on the phase difference, turbulent kinetic energy measures the distribution of velocities within a voxel and the signal drop out from turbulence (i.e. the velocity fluctuations). Using a complex algorithm to account for fluid density, turbulent kinetic energy was assessed in 3 dimensions and expressed as a peak turbulent kinetic energy.

The 4D flow sequence can be exploited to provide data on turbulent kinetic energy, as described previously.[72] The parameters were as for the standard 4D flow sequence. Although the turbulent kinetic energy sequence is not affected by aliasing, the velocity encoding was set at the lowest possible value to avoid this phenomenon as the same data were used for flow mapping. Using commercially available software (EnSight 9.2, CEI, Apex, North Carolina), visual inspection of the TKE streamlines was obtained. The TKE

data was volume rendered in the ascending aorta at each time point across a cardiac cycle. This allows visualisation of the TKE field within the area of interested and allows adjustment of the geometric constraints of the ascending aorta by manual segmentation. The aortic segment spans the valve to the top of the ascending aorta (just at the start of the aortic arch). A plot of TKE across time was produced for each subject.

This technique has been validated against particle velocimetry and fluid dynamic studies showing excellent agreement.[119] TKE has been shown to be feasible in human studies in aortic coarctation and AS with good agreement between computational fluid dynamic prediction.[120] Inter observer variability was also good in the same study.[72]

Using the same technique as the Dyverfeldt group, I analysed 10 randomly chosen datasets for intra-observer variability with a co-efficient of variation of 5.7%.

2.5.5 Whole heart 4D flow

Retrospectively gated 4D flow sequences can be used in combination with SSFP cine imaging of the LV to quantify blood flow entering and leaving the heart, as well as the kinetic energy of the moving blood within the heart at each point in the cardiac cycle, as previously published.[122] The spatial resolution is lower than for the aorta (3 x 3 x 3mm^3). Other acquisition parameters included a VENC of 100-150 cm/s, a flip angle of 8°, a TE of 3.7ms, and a TR of 6.3ms. Parallel imaging using sensitivity encoding (SENSE) with a speed-up factor of 2 was used. The background phase was corrected with a third order polynomial fit to remove phase errors arising from concomitant fields and data quality control steps were applied as previously described using automated customised Matlab

software (The Mathworks Inc., Natick, Massachusetts, USA).[121] Velocity data was converted into a file format compatible with commercially available visualisation software (EnSight, CEI Inc., Research Triangle Park, NC, USA).

Data sets were analysed using a method previously described by Eriksson *et al* with freely available software (Segment, version 1.9 R2842) which manual segments the LV endocardium from short-axis (SA) images at end diastole (ED) and end systole (ES) in a manner analogous to a standard short axis stack.[122] The segmentation at ED was resampled to give a volume with isotropic voxels equal to the size of the flow data voxels. A pathline was then emitted from the centre of each voxel included in the LV segmentation. Pathlines were created both forwards and backwards in time to the preceding and subsequent ES, respectively. These pathlines represent the complete LV end diastolic volume tracked over one cardiac cycle. The positions of all pathlines at the time of ES relative to the LV cavity (defined by the segmentation at ES) were then used to divide them into four functional flow components; direct flow, retained inflow, delayed ejection flow and residual volume as described previously.[121, 123] Direct flow is defined as blood that enters and exits the LV in the analysed cardiac cycle, retained inflow enters the LV but does not exit during the analysed cardiac cycle, delayed ejection flow starts within the LV but exits during the analysed cardiac cycle, residual volume is the component that neither enters nor exits the LV for at least 2 cardiac cycles.[121] Accuracy of this quantification was evaluated by comparing the LV inflow components (direct flow and retained inflow) to the LV outflow components (direct flow and delayed ejection flow) with any data sets with a >10% difference were excluded from further analysis.

The kinetic energy (KE) of these flow components can be calculated throughout the cardiac cycle by utilising:

$$KE = ½ \cdot \rho blood \cdot Vpathline \cdot v2pathline$$

where ρ_{blood} is blood density, $V_{pathline}$ the volume that one pathline represents and $v_{pathline}$ the velocity of the pathline at a given time point.[96] The KE for each component is the sum over all pathlines in the group. The KE values were calculated over the cardiac cycle.

A small sub-group of patients underwent whole heart 4D flow acquisition and the variability of the technique was tested within our group (I was one of the investigators) for intra and inter-observer variability suggesting good limits of variability (table 2-4).

	Intra-observer (Investigator 1)			Inter-observer (Investigator 2)	
	Analysis 1.1	**Analysis 1.2**	**CoV**	**Analysis 2**	**CoV**
Direct flow, %	38 ± 4	37 ± 5	3.9	36 ± 5	5.7
Retained inflow, % EDV	14 ± 3	15 ± 3	3.6	15 ± 3	2.6
Delayed ejection flow, % EDV	17 ± 3	17 ± 3	6.1	18 ± 3	4.8
Residual volume, % EDV	31 ± 3	30 ± 3	3.5	31 ± 4	5.0
Ejection fraction, % EDV	70 ± 3	69 ± 3	4.0	67 ± 4	4.1
EDV, ml	162 ± 46	166 ± 44	4.3	154 ± 40	5.8
ESV, ml	50 ± 16	51 ± 17	5.7	51 ± 16	5.6

Table 2-4. Intra and inter-observer variability of whole heart 4D flow. CoV Coefficient of variation; EDV End diastolic volume; ESV, End systolic volume.

2.5.6 Statistical analysis

Data were tested for normality using the D'Agostino and Pearson omnibus normality test and presented as mean ± standard deviations for parametric data and non-parametric as median and interquartile range unless otherwise specified. Linearity was assessed visually and no log transformation of data was required for those data points used in correlation analysis.

Independent two tailed t-tests were used for comparison between two groups with parametric data. Mann Whitney U tests were used for non-parametric data. With more than two groups, one way analysis of variance method was used (ANOVA). One-way ANOVA with *post hoc* Bonferroni's multiple comparisons test (parametric) or Kruskal-

Wallis H (for non-parametric data) with *post hoc* Dunn's multiple comparison tests were performed as appropriate. Correlation was assessed using the Pearson or Spearman method as appropriate. A p value of <0.05 was considered significant.

Statistical analysis was performed using SPSS (version 22, IBM, Chicago, USA). Additional statistical methods specific to the results in individual chapters are described within the appropriate methods section.

3 AORTIC SCLEROSIS: CURRENT COMMUNITY PREVALENCE AND ASSOCIATIONS

3.1 ABSTRACT

3.1.1 Background and Aims

Aortic sclerosis (ASC) is associated with hypertension and an adverse cardiovascular risk profile. I aimed to describe current community prevalence and assess the associations with hypertension and its treatment, in particular comparing the antihypertensive treatment regimes in those with and without ASC. I also compared basic transthoracic echocardiographic parameters between groups to establish if effects on the LV associated with ASC are present.

3.1.2 Methods

I used 2004 data datasets from the OxVALVE population cohort study to define the prevalence and associations of ASC. Participants were then matched for cardiovascular risk factors, including age. Any new associations with ASC were then defined using these matched data. Finally, cohorts with and without a new diagnosis of ASC were matched and compared according to the presence or absence of pre-existing hypertension (according to existing GP diagnosis).

3.1.3 Results

ASC was more common than previously recognised with a community prevalence of 38.5% in the over 65 year old population. Participants with a new diagnosis of ASC were

older (mean of 72.7 years compared with 71.8 years, p<0.001), more hypertensive (mean systolic blood pressure of 139.5mmHg compared with 138.4mmHg, p=0.001) and had a more extensive existing cardiovascular history (history of hypertension, past coronary artery bypass surgery, past myocardial infarction, hypercholesterolemia; all p<0.05). After propensity matching, subjects with ASC but without an existing diagnosis of hypertension had higher measured blood pressure (at study visit) than those without ASC (mean systolic blood pressure 4.8mmHg higher in ASC, p=0.004). There were no differences in antihypertensive prescribing in the hypertensive population with ASC compared with hypertensives without ASC; nor was their measured study visit blood pressure higher. A persistent finding across groups was a slightly thicker interventricular septum and a slightly larger LV end diastolic dimension in those with ASC.

3.1.4 Conclusion

ASC was common and associated with ageing, hypertension and a history of cardiovascular disease. However, if hypertension was recognised and treated, subjects with ASC did not have a significantly higher blood pressure than those with hypertension and no ASC, nor did they require additional classes of anti-hypertensive therapy. However, measured blood pressure was significantly higher in those without a history of hypertension than their counterparts with no ASC. A thicker septum and larger LV end diastolic diameter (implying adverse remodelling) was associated with ASC.

3.2 INTRODUCTION

Aortic sclerosis (ASC) is common, affecting around thirty percent of those over the age of 65 years.[3] While it has been considered to be the precursor of aortic stenosis (AS), the majority of participants with ASC do not progress to clinically important stenosis.[124, 125] Far from being a benign precursor of AS, it represents an adverse vascular phenotype; its presence denotes a 50% increased risk of cardiovascular death and morbidity at 5 years.[3, 124, 126, 127] This is thought to reflect, in part, ASC as a surrogate for occult coronary artery disease since the histological abnormalities of valvular endothelium are similar to atherosclerotic vascular endothelium (including coronary arteries).[29] This has led to ASC being coined a 'window to the coronary arteries'.[126] However after adjustment, atherosclerosis does not fully account for the increased risk.[3]

Some of the additional risk may relate to the higher prevalence of hypertension in ASC.[3, 128, 129] There are theoretical reasons why hypertension is prevalent and may be more resistant to therapy. Small studies have demonstrated impaired response to nitric oxide in ASC, indicating endothelial abnormalities that increase vascular stiffness.[130-132] Whether this a primary phenomenon related to ASC, or whether the changes reflect hypertension, is unknown. Indeed, despite the high incidence of ASC in the population, very little evidence exists concerning best management. This paucity of evidence is probably due to some basic, yet important obstacles. First is a lack of consensus agreement on the definition of ASC. For example, maximal transvalvular gradient is defined as less than 2.5m/s in some studies and less than 2 m/s in others.[3, 124, 133] Some, including the major Cardiovascular Health Study, have used both definitions at different

time points within the study.[3] The diagnosis of leaflet thickening, with or without calcification, is subjective and also depends on the quality of the echocardiographic study (particularly the parasternal views), as well as the settings used (in particular the harmonic and gain settings). Echocardiographic technology has improved rapidly, affording much improved image quality in newer studies. ASC may, in the early stages, may be valvular thickening without calcification. However, most but not all, studies have included in their diagnosis, evidence of calcification; notable exceptions of studies which did not demand calcification include the LIFE study and follow up data from Brazil of an inpatient population.[134, 135] This has further challenges for diagnosis and quantification and may be missed with challenging echo views, or CT, which relies on Agatston scoring of calcium.[125, 129, 136] Attempts to quantify ASC, for example using backscatter, rely on calcification on the valve, which may miss those with predominantly thickened but non-calcified valves.[137] Thus, the reported prevalence of ASC varies widely in existing literature.[4] Furthermore, as there is no proven management strategy for ASC (either as a valvular or vascular phenotype), it is often unreported in studies and may not be seen as a fruitful area for research, for some of these reasons.

To date, there are no contemporary prospective studies with the primary aim of ascertaining the prevalence of valvular heart disease in a rapidly aging population. Much of our understanding of the prevalence and associations of ASC comes from data from the early 1990's Cardiovascular Health Study, using old echocardiographic technology and variable definitions of ASC. Therefore, up to date prevalence studies in a UK population are warranted to define the prevalence and current associations of ASC. Using data from the OxVALVE Population Cohort Study (OxVALVE-PCS), this chapter describes the clinical,

pharmacological and echocardiographic phenotypes of participants with a new diagnosis of ASC.

I hypothesised that a diagnosis of hypertension was more prevalent in ASC and that even once those with hypertension with and without ASC are matched, the absolute value of the measured blood pressure would be higher in those with ASC (secondary to endothelial abnormalities and increased aortic stiffness). I explored if there are differences in prescribing patterns of antihypertensive agents between those with and without ASC and suggest that hypertension was more resistant to therapy in the context of ASC.

3.3 METHODS

OxVALVE-Population Cohort Study (OxVALVE-PCS) began recruiting in Oxfordshire in 2009. Its primary aim was to establish well phenotyped population cohorts of participants with previously undiagnosed valvular heart disease for longitudinal follow up. Participants were recruited through one of five participating general practice surgeries in Oxfordshire. Those with known valve disease, terminal illness, immobility, frailty (as judged by the general practitioner, including dementia and housebound participants) were excluded. Participants were invited by letter, with a single follow-up reminder sent to non-responders. The detailed methods for this study have been published previously.[100]

For those declared as ASC or without VHD, the following methods are relevant. Echocardiography was performed by the British Society of Echocardiography (BSE) accredited sonographers or physicians using a Vivid-Q portable machine (Vingmed-General Electric, Horton, Norway). A two-beat clip was stored for each image unless in atrial fibrillation where a five-beat clip was acquired. Measurements were not recorded immediately following an ectopic beat. When assessing regurgitation with colour flow Doppler, the Nyquist limit was set to 50–60cm/s. In addition to standard acquisition protocols, the following images/measurements were obtained:

- Zoomed images of the aortic valve, aortic root and ascending aorta, along with any regurgitation (parasternal long and short axis).
- Continuous wave Doppler through aortic, mitral and tricuspid valves to demonstrate presence/absence of any regurgitation (apical five/four/three and two-chamber views).

Left ventricular ejection fraction was assessed using the Teichholz calculation if left ventricular systolic function was judged qualitatively normal by an experienced operator. When used in this context, Teichholz calculation performs well.[138] Visual assessment, is also accurate if done by experienced practitioners.[139] Abnormal systolic function led to formal biplane volumetric assessment of ejection fraction, classified as: normal ≥55%, mildly impaired 45–55%, moderately impaired 35–44% and severely impaired <35%.

Data from the first 2500 participants from OxVALVE-PCS were analysed to assess the prevalence of ASC, associated comorbidities and the prescription of anti-hypertensive and other cardiovascular medication. Participant demographic and echocardiographic data

were analysed. Participants with more than mild other valve disease were excluded from the analysis (whether ASC was present or not), resulting in 2004 participants for this analysis.

A basic medical and medication history was taken. Height, weight and blood pressure were obtained. A standard transthoracic echocardiographic examination was undertaken by BSE accredited sonographers or physicians, using GE Vivid Q machine (GE Healthcare, Waukesha, WI, USA) according to the British Society of Echocardiography (BSE) standard transthoracic dataset. Additional images with particular attention to the heart valves, including an optimised, zoomed image of the short axis and long axis views of the aortic valve in the parasternal positions were acquired. From these images, ASC was defined based on European Association of Echocardiography/American Society for Echocardiography guidelines as 'thickening and focal calcification of the aortic valve leaflets, with normal or near normal cusp mobility and with maximum aortic valve transvalvular velocity less than or equal to 2.5m/s.[140] To ensure quality control and reduce intra observer variability, at least 20% of all studies were co-reviewed and a consensus diagnosis used in difficult cases.

The general practice coding definition of hypertension and hyperlipidaemia was used. Hypertension was defined as a blood pressure reading of greater than 140/90mmHg on more than one occasion (this result is Read coded in general practice in England and Wales as 'hypertension'). The absolute blood pressure refers to that taken at the time of the study visit.

Participants were stratified by age and gender to explore the prevalence of VHD in the study population and by presence or absence of ASC to examine its demographic and clinical characteristics. Descriptive statistics are presented using means and standard deviations (SD) for continuous variables and counts (percentages) for categorical variables. Student's t-test and Chi-squared test or Fisher's exact test were used to explore dependencies between valve disease and quantitative and categorical variables, respectively.

The matched cardiovascular variables were current smoking, history of hypertension, hyperlipidaemia, myocardial infarction, stroke or transient ischaemic attack, diabetes mellitus, coronary artery bypass grafting and percutaneous coronary intervention. The participants were matched using the psmatch2 module version 4.0.6 in Stata SE 12.1 (College Station, Texas) with probit regression based generation of propensity scores, and 1-1 nearest neighbour matching with a maximum radius of 0.01. This gave 653 matched pairs - 76 ASC cases could not be matched effectively. All results are expressed as means ± standard deviations, with a 2-tailed p-value <0.05 considered significant. The study was approved by the Southampton Regional Ethics Committee and all participants provided written informed consent.

3.4 RESULTS

3.4.1 Main demographic findings (Unmatched data-Table 3-1)

Using unmatched data from the total population, ASC was a common finding in the over 65 year old population without known VHD, with an overall prevalence of 38.5%, rising

with each decade to affect over 60% of the over 85 year old age group (Figure 3-1). Those with ASC were older by a mean of 2.45 years. No gender difference was found.

3.4.2 Associations with existing cardiovascular disease (unmatched data-Table 3-1)

Unmatched data demonstrated an association with hypertension (53.4% of those with a new diagnosis of ASC, compared with 41.3% of those without ASC; $p<0.001$). Other statistically significant cardiovascular associations were found with a history of myocardial infarction, past coronary bypass surgery, hyperlipidaemia and cerebrovascular accident. No association was found with diabetes, percutaneous coronary intervention (PCI) or atrial fibrillation (AF). Those with ASC were more likely to be prescribed antihypertensive agents, statins, diuretic therapy and aspirin.

3.4.3 Echocardiographic findings in unmatched population (unmatched data-Table 3-1)

In ASC, the left atrium (LA) diameter was larger, the inter-ventricular septum (IVS) thicker and the LV end diastolic dimension (LVEDD) larger. No difference in ejection fraction (EF) was found.

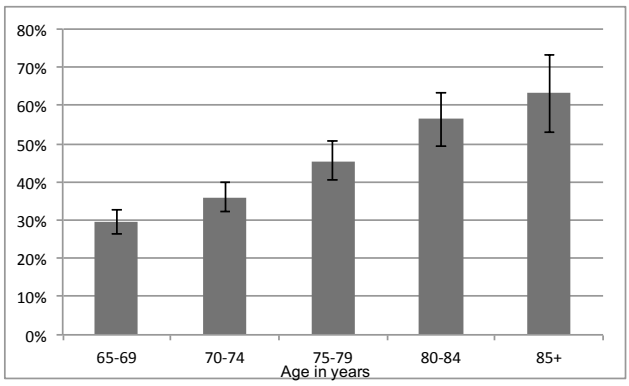


Figure 3-1. Prevalence of aortic sclerosis by age in years (error bars represent standard deviation).

	All participants (N=2004)	No aortic sclerosis (before matching, N=1231)	Aortic sclerosis (before matching, N=773)	P value
	Number (%) or Mean (SD)	**Number (%) or Mean (SD)**	**Number (%) or Mean (SD)**	
Demographics				
Age	72.71(5.79)	71.77(5.26)	74.22(6.25)	<0.001
Gender (male)	994(49.6%)	612(49.7%)	382(49.4%)	0.89
Hypertension	921(46.0%)	508(41.3%)	413(53.4%)	<0.001
MI	105(5.2%)	51(5.4%)	54(7.0%)	0.005
CVA	119(5.9%)	60(4.9%)	59(7.6%)	0.011
Smoker	153(7.6%)	153(7.6%)	52(6.7%)	0.225
CABG	37(1.8%)	37(1.8%)	22(2.8%)	0.008
Diabetes	240(12.0%)	143(11.6%)	97(12.5%)	0.532
Hyperlipidaemia	719(37.6%)	413(35.1%)	306(41.6%)	0.004
PCI	78(3.9%)	42(3.4%)	36(4.7%)	0.161
Atrial Fibrillation	61(3.0%)	35(2.8%)	26(3.4%)	0.359
Medications				
Mean number of class of antihypertensive drugs	0.83 (1.0)	0.74 (1.0)	0.97 (1.0)	<0.001
ACEI/ARB	683(34.1%)	372(30.2%)	331(40.2%)	<0.001
B Blockers	299(14.9%)	171(13.9%)	128(16.6%)	0.103
Ca Channel Blocker	312(15.6%)	171(13.9%)	141(18.2%)	0.009
Diuretic	363(18.1%)	192(15.6%)	171(22.1%)	<0.001
Lipid lowering agent	763(38.1%)	435(35.3%)	328(42.4%)	0.001
Aspirin	434(21.7%)	240(19.5%)	194(25.1%)	0.003
Other antiplatelet	30(1.5%)	16(1.3%)	14(1.8%)	0.509
Examination				
Heart rate beats/min	73.22(11.86)	73.93(11.87)	72.09(11.75)	0.0007
Systolic BP mmHg	139.50(19.19)	138.42(19.25)	141.22(18.97)	0.0014
Diastolic BP mmHg	79.34(11.40)	79.81(11.49)	78.60(11.23)	0.022
BMI kg/m^2	27.9(5.0)	27.9(4.9)	27.9(5.1)	0.97
Echo findings				
LA cm	3.68(0.62)	3.64(0.59)	3.73(0.66)	0.0008
IVS cm	0.99(0.23)	0.97(0.22)	1.04(0.25)	<0.001
EF%	67.5(8.35)	67.63(7.69)	67.28(8.93)	0.359
LVEDD cm	4.57(0.69)	4.55(0.62)	4.61(0.79)	0.039

Table 3-1. Main results from unmatched population. Results are presented as mean (SD) for continuous variables and numbers (%) for categorical variables. *P-value is for t-test (continuous) and χ^2 (categorical) for each characteristic/condition.

3.4.4 Comparison of matched data (Table 3-2)

Participants were matched for age, gender and cardiovascular history, including a history of hypertension. No difference was found in the prescribing pattern of cardiovascular medications between groups. However, the absolute systolic blood pressure, as measured at the study visit, was higher in those with ASC (140.6mmHg compared with 137.4mmHg, p=0.002), a mean difference of 3.1mmHg. A small increase in thickness of the IVS (1.03cm compared with 0.99cm; p=0.002) and a slightly larger LVEDD (4.64cm compared with 4.48cm; p <0.001) were found in ASC. No difference was found in ejection fraction (EF), left atrial diameter (LA) or diastolic blood pressure.

	No ASC (N=653)	ASC (N=653)	P value*
Matched variables			
Age	73.33(5.82)	73.11(5.45)	0.07
Gender (male)	322(49.3%)	322(49.3%)	1.00
Hypertension	327(50.1%)	337(51.6%)	0.415
MI	34(5.2%)	44(6.7%)	0.253
CVA	42(6.4%)	49(7.5%)	0.804
Smoker	47(7.2%)	49(7.5%)	0.88
CABG	15(2.3%)	13(2.0%)	0.728
Diabetes	80(12.3%)	76(11.6%)	0.728
Hyperlipidaemia	263(40.3%)	273(41.8%)	0.423
PCI	29(4.4%)	32(4.9%)	0.788
Medications			
Mean number of class antihypertensives	0.87 (1.0)	0.94 (1.02)	0.14
ACEi/ARB	235(36.0%)	254(38.9%)	0.213
Beta Blocker	114(17.5%)	105(16.1%)	0.546
Calcium Channel Blocker	99(15.2%)	119(18.2%)	0.135
Diuretic	119(18.2%)	134(20.5%)	0.303
Lipid lowering agent	270(41.3%)	284(43.5%)	0.316
Aspirin	161(24.7%)	157(24.0%)	0.834
Other antiplatelet	21(3.2%)	12(1.8%)	0.832
Examination			
Heart rate beats/min	73.95(12.33)	72.16(11.85)	0.009
Systolic BP mmHg	137.38(18.53)	140.56(18.87)	0.002
Diastolic BP mmHg	78.88(11.54)	78.40(10.99)	0.446
BMI kg/m^2	27.8(4.8)	27.6(4.7)	0.37
AF	10(1.5%)	21(3.2%)	1.00
Echo findings			
LA dimension cm	3.69(0.62)	3.72(0.64)	0.271
IVS cm	0.99(0.23)	1.03(0.24)	0.0016
EF%	67.36(8.01)	67.49(9.05)	0.783
LVEDD cm	4.48(0.58)	4.64(0.82)	0.00002

Table 3-2. Matched Data for age, gender and cardiovascular disease. Results are presented as mean (SD) for continuous variables and numbers (%) for categorical variables, with percentages denoting proportion within each group. *P-value is for paired t-test (continuous) and McNemar test (categorical) for each characteristic/condition.

3.4.5 Sensitivity analysis between those with hypertension with and without ASC (matched data Table 3-3)

Data were further sub-divided to compare matched participants with (Table 3-3) and without (Table 3-4) a diagnosis of hypertension.

This analysis was performed to elicit differences in the study clinic recorded blood pressure between groups to ascertain if those with ASC had higher blood pressure or differences in prescribing patterns of cardiovascular medication, especially antihypertensive agents.

In those with a prior diagnosis of hypertension, no differences were found between absolute blood pressure with and without the presence of ASC (systolic blood pressure 141.2mmHg compared with 140.7mmHg; p 0.24). The mean number of antihypertensive agents in each group was 1.6 (p=0.51). There were no differences in the choice of antihypertensive agent or in prescribing rates for other cardiovascular medication. The echocardiographic data showed a small but statistically significant increase in IVS (1mm) and LVEDD (2mm) in ASC. No differences in EF or LA diameter were seen.

3.4.6 Sensitivity analysis between those without hypertension with and without ASC (matched data Table 3-4)

In those without a history of hypertension, those with ASC had a higher systolic blood pressure than those with a normal aortic valve (138.6mmHg compared with 134.4mmHg; p=0.004). There was no difference in prescribing patterns of cardiovascular medication and no difference in the LA diameter, IVS, or EF, although subjects with ASC had a larger ventricle (LVEDD 4.6cm compared with 4.4cm, p= 0.02).

	After matching		
	No ASC (N=335)	**ASC (N=335)**	**P value**
Matched variables			
Age	74.0 (5.8)	73.9 (5.8)	0.47
Female	163 (48.7%)	163 (48.7%)	1
MI	29 (8.7%0	27 (8.1%)	0.89
CVA/TIA	31 (9.3%)	34 (10.1%)	0.78
Current smoker	15 (4.5%)	20 (6.0%)	0.44
CABG	9 (2.7%)	9 (2.7%)	1
Diabetes	58 (17.3%)	58 (17.3%)	1
Hyperlipidaemia	175 (52.2%)	178 (53.1%)	0.85
PCI	19 (5.7%)	24 (7.2%)	0.51
Medications			
Mean number of class of antihypertensive drugs	1.6 (0.9)	1.6 (0.9)	0.51
ACEi or ARB	223 (66.6%)	231 (69.0%)	0.57
Beta blocker	95 (28.4%)	88 (26.3%)	0.61
Calcium channel blocker	96 (28.7%)	104 (31%)	0.54
Diuretic	120 (35.8%)	126 (37.6%)	0.69
Lipid lowering agent	184 (54.9%)	190 (56.7%)	0.67
Aspirin	114 (34.0%)	105 (31.3%)	0.5
Other antiplatelet	11 (3.3%)	9 (2.7%)	0.81
Examination			
Systolic BP mmHg	140.7 (19.8)	142.5 (18.6)	0.24
Diastolic BP mmHg	79.4 (12.5)	78.7 (11.6)	0.47
Heart rate beats/min	73.4 (12.5)	71.5 (12.3)	0.05
BMI kg/m^2	28.5 (4.8)	29.3 (5.2)	0.04
AF	12 (3.6%)	12 (3.6%)	1
Echo findings			
LA dimension cm	3.8 (0.7)	3.9 (0.6)	0.1
IVS cm	1.00 (0.2)	1.09 (0.3)	<0.0001
EF%	67.4% (8.6%)	68.3% (9.6%)	0.17
LVEDD cm	4.54 (0.6)	4.70 (0.9)	0.007

Table 3-3. Matched data for hypertensive subjects. Results are presented as mean (SD) for continuous variables and numbers (%) for categorical variables, with percentages denoting proportion within each group. *P-value is for paired t-test (continuous) and McNemar test (categorical) for each characteristic/condition.

	After matching		
	No ASC (N=309)	**ASC (N=309)**	**P value**
Matched variables			
Age	72.6 (5.5)	72.6 (5.5)	0.74
Female	158 (51%)	158 (51%)	1
MI	6 (1.9%)	6 (1.9%)	1
CVA/TIA	6 (1.9%)	10 (3.2%)	0.3
Current smoker	27 (8.7%)	27 (8.7%)	1
CABG	0 (0%)	0 (0%)	1
Diabetes	17 (5.5%)	20 (6.5%)	0.69
Hyperlipidaemia	92 (29.8%)	78 (25.2%)	0.02
PCI	5 (1.6%)	4 (1.3%)	1
Medications			
Mean number of class of anti-hypertensive drugs	0.1 (0.4)	0.1 (0.4)	0.48
ACEi or ARB	15 (4.9%)	16 (5.2%)	1
Beta blocker	14 (4.5%)	15 (4.9%)	1
Calcium channel blocker	2 (0.6%)	9 (2.9%)	0.07
Diuretic	6 (1.9%)	4 (1.3%)	0.75
Lipid lowering agent	90 (29.1%)	77 (24.9%)	0.14
Aspirin	44 (14.2%)	43 (13.9%)	1
Other antiplatelet	2 (0.6%)	1 (0.3%)	1
Examination			
Systolic BP mmHg	134.4 (17.4)	138.6 (18.6)	0.004
Diastolic BP mmHg	78.5 (11.0)	78.7 (10.3)	0.87
Heart rate beast/min	74.2 (11.4)	72.7 (10.9)	0.09
BMI kg/m^2	26.5 (4.4)	26.4 (4.2)	0.72
AF	9 (2.9%)	7 (2.3%)	0.8
Echo findings			
LA dimension cm	3.6 (0.6)	3.6 (0.6)	0.4
IVS cm	0.97 (0.3)	0.98 (0.2)	0.45
EF%	67.2 % (8.2%)	67.2% (7.5%)	0.96
LVEDD cm	4.44 (0.6)	4.55(0.7)	0.02

Table 3-4. Matched data for non-hypertensive subjects. Results are presented as mean (SD) for continuous variables and numbers (%) for categorical variables, with percentages denoting proportion within each group. *P-value is for paired t-test (continuous) and McNemar test (categorical) for each characteristic/condition.

3.5 DISCUSSION

3.5.1 Main findings

Our results show that ASC is common in the over 65 year old population without known VHD, affecting around 38%, with a progressively higher prevalence according to age - ultimately affecting over 60% of those over 85 years. ASC is also strongly associated with a history of cardiovascular disease and with hypertension. ASC participants were more likely to be prescribed antihypertensive medication and other cardiovascular drugs. Contrary to my hypothesis, I found no evidence that participants with a history of hypertension and ASC have a significantly higher measured blood pressure or differences in the prescription of anti-hypertensive medication. However, those without a history of hypertension have a higher blood pressure as measured at the clinic visit.

3.5.2 Prevalence comparison with other studies

A higher prevalence of ASC was found compared with previous echocardiographic studies. The reasons for this are likely to reflect the use of modern echocardiographic equipment, the primary aim of studying valve disease (with associated investigator bias), and enrolment of an older study population.

Study	Population	Mean Age in years	Definition ASC	Prevalence ASC	Associations
CVHS (2007) [124]	5621 community dwellers in North America (data collected 1989-1992)	72.9±5.5	Varied. Valve thickening with velocity <2.5m/s. Latter recruitment used <2m/s	29%	Male gender, age, hypertension, coronary artery disease, renal insufficiency
Monica Kora (2009)[128]	953 random selection of German participants (data collected 1994-1995)	57.7±11.7	Calcification or thickening but no velocity specified (mean velocity 1.51m/s)	28% prevalence of aortic valve degeneration	Age, hypertension, obesity, hypercholesteroleamia, prior cardiovascular disease, diabetes
SHIP study (2008)[141]	2081 German subjects free of cardiovascular disease and cancer (data collected around 2001)	60 years (range 53-69)	Calcification and thickening without reduced systolic opening	25.4%	Age, male gender, hypertension, hypercholestolaemia, diabetes.
SPARC study (2001)[142]	381 Randomly selected American subjects (data collected late 1990s)	67 (range 51-101)	Valve thickening	35.4%	Age, male gender, diabetes, hypertension, body mass index, use of cholesterol lowering agents

Table 3-5. Summary of major epidemiological studies of aortic sclerosis.

The largest study of aortic valve disease comprises data from the Cardiovascular Health Study (CVHS), a study of 5621 community dwellers in North America.[3, 124] Although primarily designed to be a longitudinal study of coronary heart disease and stroke, sub-studies have included baseline and follow up data for ASC.[143] The prevalence of ASC was 29% with a mean age very close to our population of 72.9 ± 5.5 years. Baseline associations with male gender, age, hypertension, coronary disease and renal insufficiency were found. In common with our study, no association with diabetes was detected. However, a cut off value of <2.5m/s transvalvular velocity was used for the original study, and a sub group of 687 African-American subjects added using a lower cut off of <2m/s. The baseline data were collected in 1989-1990, with additional recruitment

of African-American participants between 1990-1992. It is likely that the newer echocardiographic equipment available for OxVALVE-PCS, more than two decades later, increased the sensitivity of ASC detection and accounts for at least some of the increased prevalence.

The Monica Kora study, conducted in Northern Europe, enrolled 953 subjects in 1994-1995 and reported in 2009.[128] It used echocardiography to study randomly selected German participants with a mean age of 57.7 ± 11.7 years and was designed primarily to detect trends and determinants of cardiovascular disease. The study classified ASC amongst 'degenerative aortic valve disease', defined as calcification or thickening on echocardiography but without providing an upper cut off velocity. The mean transvalvular velocity was 1.51m/s, so most participants would be classified in keeping with ASC. Direct parallels cannot be drawn with this study but the implication is that most of these participants had early disease. The prevalence of aortic valve degeneration in this younger age group was 28% (measured at the baseline study in 1994/1995). When age specific groups were studied, the prevalence was much more in keeping with our findings; 38% of those aged 65-74 years and 64% of those aged 75-84 years. Baseline associations with age, hypertension, obesity, hypercholesterolaemia and prior cardiovascular disease were found. The authors also described a significant association with diabetes. No gender differences were seen. Statins, ACE inhibitors, beta blockers and anti-platelet agents were prescribed more frequently in ASC subjects.

The SHIP study, also conducted in Germany, reported in 2010[141] and followed up 2081 participants over 8 years to establish if aortic and mitral valve calcification predicted

cardiovascular events. Baseline data gave a prevalence of ASC of 25.4% with mean age 60 (range 53-69) years. Strong associations were found with age, male gender, hypertension and, in common with Monica Kora, diabetes. Subjects with ASC were more likely to be prescribed antihypertensive agents and lipid lowering therapy. It may be that diabetes predisposes to ASC in these younger populations, but this relationship is lost in the older age group, or masked by concurrent hypertension, as neither the CHVS study nor OxVALVE-PCS report this association.

3.5.3 Associations and links with hypertension

The association with ASC and hypertension is well established and reported in major studies. Both CHVS and Monica Kora defined hypertension in a very similar way to OxVALVE-PCS: systolic blood pressure >140mmHg, diastolic blood pressure >90mmHg or those receiving prescriptions for antihypertensive medication. OxVALVE-PCS reports a 41.3% prevalence of hypertension without ASC and a 53.4% prevalence of hypertension with ASC. The CHVS recorded slightly higher levels of hypertension overall, possibly reflecting local differences, including racial mix; 56% hypertension in those without ASC compared with 67% in ASC. Monica Kora recorded hypertension in 32% without ASC vs 50% with ASC. The SHIP study quoted mean systolic/diastolic blood pressure of 138/86mmHg in the non-ASC and 147/84mmHg in those with ASC. This group difference may be more marked as the population was younger (maximum age 69 years). However, the response to treatment and blood pressure measurements in those with recognised hypertension and ASC was not been studied.

3.5.4 Comparison of cohorts with and without hypertension and ASC

The hypothesis of this chapter is that hypertension in subjects with ASC may be more resistant or difficult to treat than in those with hypertension without ASC, reflecting the increased vascular stiffness in ASC compared with age-matched controls (as demonstrated using a non-invasive assessment of pulse transit time and ankle brachial index).[144, 145] Pulse wave velocity, measured by transthoracic echocardiography has also been shown to be faster, implying increased arterial stiffness[146] and a reduced response to nitric oxide has been demonstrated by impaired flow-mediated dilatation.[16] Moreover, an impaired response to nitric oxide has been demonstrated in patients with ASC using basic science techniques (tissue response to nitric oxide and plasma concentrations of asymmetric dimethylarginine, a marker of endothelial function) linked with impaired flow-mediated dilatation in the same subjects.[130, 131]

The data from OxVALVE-PCS support the strong association with hypertension. A higher systolic blood pressure (mean difference 3.2mmHg, p= 0.004) was found in participants with ASC but without pre-existing hypertension. However, in the group with prior hypertension, the mean difference dropped to 1.8mmHg and statistical significance was lost, suggesting that there is no important resistance to therapy once hypertension is recognised. There were no differences in the number of antihypertensive agents used or the patterns of primary care prescribing. However, I detected a signal that adverse remodelling may occur - the IVS was thicker in ASC by a mean of 1mm (IVS diameter 1.1cm). Although this difference is very small, it is strongly significant ($p<0.001$), suggesting a real population difference. It is difficult to fully understand what this may mean in terms of prognosis since evidence is conflicting. Based on the ASE and ESC joint

guidelines, a septal thickness of 1.1cm is mildly abnormal in both genders (but not the British Society of Echocardiography guidelines which include up to 1.2cm as normal).[140] There are no clear cut mortality data relating to septal thickness in healthy populations but studies estimating mass or measuring septal thickness in hypertension found a stepwise increase in mortality with LV hypertrophy.[147, 148, 149] In established coronary disease, even a mild increase in septal thickness is associated with adverse prognosis.[150, 151]

The driver of increased mortality in ASC may not, as hypothesised, be related to difficult to control hypertension or under-treatment. However, adverse remodelling may suggest that there is more to the phenomenon of hypertension and ASC than simple blood pressure measurements and that ASC may not be haemodynamically benign. My data show some features of LV remodelling with a consistently larger LVEDD, accompanied in all groups (except those without hypertension) by a slightly thicker IVS. An increase in stroke volume may raise arterial blood pressure, although whether this is a chronic adaption which is seem at rest is unclear, for example, males with larger hearts do not typically have a higher blood pressure than females with a lower stroke volume, in the absence of pre-menopausal hormonal differences.[152] It is possible that the larger ventricle may relate to additional afterload arising from arterial stiffness although this change might then also be expected in participants with hypertension but no ASC.

It is possible that minor valve changes impact the LV in ways immeasurable by standard transthoracic echocardiography. Flow through abnormal valve leaflets creates turbulence. This in turn causes irreversible energy transformation as heat and

consequent pressure loss. So, kinetic energy conservation may still be impaired despite a normal valve area. The degree to which turbulent kinetic energy is formed in ASC has not been studied, nor have the possible effects on the LV. In the following chapters, aortic distensibility, turbulent kinetic energy and flow patterns in the ascending aorta of patients with aortic valve calcification (including a subset with ASC from OxVALVE-PCS) will be studied. LV function and geometry will be analysed to determine if there is any impact on more subtle measures of function.

3.6 LIMITATIONS

In common with any population-based study, OxVALVE PCS may be subject to selection bias, with a fitter group of participants who are more motivated towards their health responding. The study achieved a good response rate of just over 50%, which mitigates this to some extent. The deliberate exclusion of the cognitively impaired or housebound may also influence the data, as a higher incidence of valve pathology occurs in the very elderly and frail.[153] The inherently subjective nature of diagnosing ASC could be a problem but around 20% of studies were co-reviewed to reduce this.

3.7 CONCLUSIONS

Aortic sclerosis is common in community dwellers over the age of 65 years. This patient population are at a distinct adverse cardiovascular risk. The hypothesis that this is related to adverse blood pressure control is not proven when groups are closely matched for age, gender, cardiovascular risk factors and an existing diagnosis of hypertension. However,

the increased systolic blood pressure in those without a prior diagnosis of hypertension may lead to definite evidence of adverse remodelling. Whether this accounts for the additional cardiovascular risk seen in participants with ASC can only be resolved by longitudinal follow up of this cohort.

4 AORTIC GEOMETRY, FUNCTION AND FLOW

4.1 ABSTRACT

4.1.1 Background and Aims

The afterload afforded by aortic stiffness and pressure loss secondary to the formation of turbulent kinetic energy in the aorta are important additional contributors to afterload and outcome in AS. In this chapter, I hypothesised that BAV and TAV AS were associated with similar impairment of aortic stiffness (due to relative aortic dilatation and aortic stiffening respectively) whilst bicuspid AS was associated with additional energy loss as a result of more turbulent kinetic energy transformation to heat (due to a larger aorta and abnormal flow patterns). Subjects with BAV AS would therefore demonstrate less 'efficient' conservation of energy for a given valve area. Additionally, I hypothesised that abnormal flow and aortic wall shear stress would differ between tricuspid and BAV AS, which may have an impact on aortic size. Finally, as discussed in Chapter 3, ASC is not benign with similar adverse aortic stiffness to TAV AS and early valve changes will create more turbulence than in healthy volunteers.

4.1.2 Methods

89 participants (controls 23, ASC 20, TAV 22, BAV 24) were recruited and scanned using 4D flow aortic sequences and non-invasive assessments of pulse waveform and central blood pressures.

4.1.3 Results

Markers of aortic stiffness and advanced non-invasive pulse wave analysis did not show important differences between ASC and TAV AS. Subjects with ASC have stiffer aortas than controls (higher augmentation pressure by a mean of 6.6mmHg, p=0.004 and faster pulse wave velocity, p=0.0004) and significantly more turbulent kinetic energy formation (6.6mJ, compared with 3.9mJ, p <0.001). After age adjustment, pulse wave velocity remained significantly higher in ASC than in controls. Distensibility of the ascending aorta was not different between BAV and TAV AS. A larger ascending aortic diameter in BAV AS was seen compared with other groups, including TAV AS (p<0.001). Peak turbulent kinetic energy was higher in the BAV AS population than in TAV AS (p<0.001) but bivariate correlation suggests that the larger aorta is not the complete explanation for this (r=-0.46, p=0.10). Wall shear stress and flow angle were higher in AS as a whole than in ASC or controls (p=all <0.001), but with no difference between BAV or TAV AS. The site of maximal in plane WSS was different however, in TAV AS it is a more posterior peak and in BAV AS a more anterior peak.

4.1.4 Conclusion

ASC was associated with increased pulse wave velocity and higher turbulent kinetic energy formation in the aorta than in controls, implying that it is more than a condition of benign valve thickening and affects aortic stiffness. The ascending aorta in BAV AS was larger than in other groups and more turbulent kinetic energy is formed than in valve-area matched TAV AS; this does not correlate closely with the area of the ascending aorta and reasons for this remain elusive. WSS and flow angle did not differ significantly

between BAV and TAV, although the site of peak WSS was different, suggesting aortic dilatation may be more than just a flow mediated phenomenon. Exploration of the possible effects on LV function will be discussed in the following chapter.

4.2 INTRODUCTION

Outcomes in AS depend not only on the degree of stenosis at valvular level but the additional afterload from the aorta and systemic circulation.[57, 154, 155] Together, these factors create the total afterload that the LV must overcome.[155] Individual components, such as the presence of myocardial disease, patterns of LV remodelling, cardiovascular fitness and physiological demands, and body size and age interact to a greater or lesser extent in individual patients. However, in the context of a normal myocardium, broad themes emerge. Firstly, the stiffness of the aorta; measurable using non-invasive applanation tonometry or imaging based measures of aortic stiffness (central wave propagation or pulse wave velocity and aortic distensibility). Secondly, the size of the aortic root and the differential between valve area and root area which affect the conservation of kinetic energy and relate to prognosis.[69, 71] This has been recognised in catheter based assessments of cardiac output as 'pressure recovery', the phenomenon whereby static energy is converted back to kinetic energy.[156] The converse applies in larger roots when turbulent kinetic energy is converted to heat and irreversible pressure loss occurs. This can now be assessed directly using CMR measurement of peak turbulent kinetic energy with a promising new sequence developed by Dyverfeldt *et al* and validated against catheter and echocardiographic measures.[72] Since BAV AS is associated with a larger ascending aorta, more turbulent kinetic energy (and hence an increased pressure loss) may occur for a given valve area.

Debate continues as to the extent to which BAV aortopathy is related to abnormal aortic wall shear stress (WSS) as a result of abnormal flow and how much is due to an inherent aortopathy.[15, 20, 26] Recent work from our unit and others demonstrated that valve fusion patterns are associated with different adverse flow profiles and degrees of dilatation.[15] While these are different from healthy volunteers, no data exist to demonstrate how TAV AS affects aortic flow or aortic WSS. Understanding this question may shed more light on the 'nature / nurture' debate concerning BAV aortopathy.

In this chapter, I hypothesised that TAV and ASC share an adverse aortic stiffness profile. I hypothesised that regional ascending aortic stiffness occurs in BAV AS due to an abnormally expanded aortic wall. I hypothesised that flow patterns and WSS differ between TAV and BAV AS due to differences in valvular and aortic root anatomy and that BAV AS has higher turbulent kinetic energy due to a larger aorta.

As previously indicated, I do not believe that ASC is haemodymically benign. Indeed, it is possible that an adverse vascular profile and early impairment of energy conservation (measured as turbulent kinetic energy formation) is an important factor underlying the significantly poorer outcomes in this condition.

4.3 METHODS

A total of 89 participants gave informed consent to CMR scanning, as described in Chapter 2.

Indices of aortic function were assessed using SPSS with the following parameters: TR 3ms, TE 1.4ms, field of view read 380mm, in-plane resolution 1.97mm, and slice thickness 7mm. The aortic cine images were piloted perpendicular to the orientation of the aorta at the level of bifurcation of the main pulmonary artery. Immediately prior to image acquisition, blood pressure and central pressure with arterial wave-form data were acquired using Vicorder applanation tomography, calibrated to a brachial cuff, as previously described.[55]

Aortic distensibility (the fractional increase in aortic area as a function of pressure change) was calculated using automated measurements of aortic luminal area, as previously described in Chapter 2.[113] Pulse wave velocity was computed using in-house software and a widely accepted active contour model, previously validated by Herment *et al*.[157] The aortic centre line was measured from an oblique-sagittal HASTE slice (half-Fourier single-shot turbo spin-echo scan sequence) and transit time between normalized flow velocity curves was estimated using the half-maximum of the linear fit on the systolic upslope.

WSS was calculated using the 3D flow vector and magnitude data, as described by Stalder *et al*.[116] Systolic WSS (measured in Newton per square metre [N/m2]) was measured in 12 anatomic positions around the aortic lumen and used to derive the circumferentially averaged systolic WSS (WSSmag), which is the combined vector magnitude of both through-plane and in-plane (rotational) WSS.[15]

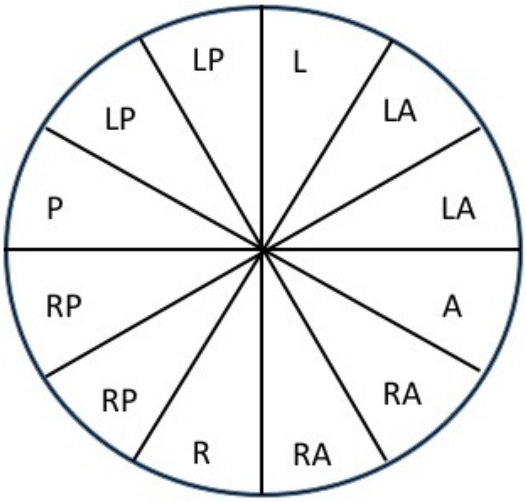


Figure 4-1. Segmentation of the aorta. L stands for left, R for right, A for anterior, P for posterior. The 12 segment divisions allows localisation of points of higher or lower shear stress

The systolic flow angle is the angle between the line perpendicular to the short axis analysis plane and the instantaneous mean flow vector at peak systole.[20] Here it is measured at the level of the mid ascending aorta, at the level of the pulmonary artery.

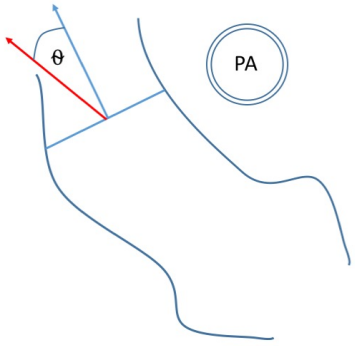


Figure 4-2. Perpendicular assessment of flow angle at the level of the pulmonary artery. Flow angle (θ) at level of mid ascending aorta. (PA = Pulmonary artery)

4.4 RESULTS

4.4.1 Participant characteristics

Participants with BAV AS were a mean of 7.9 years younger than their TAV counterparts, an anticipated finding since BAV tend to present a mean of 20 years younger.[31] Healthy volunteer controls were well matched with the BAV AS group, with ASC and TAV AS matched for age. All groups were matched for gender. No significant differences in mean body surface area existed between groups. TAV AS had more severe stenosis than BAV AS but this difference did not reach statistical significance.

	CONTROLS (23)	ASC (20)	TAV (22)	BAV (24)	P value (ANOVA)
Age (yrs)	64.2 ± 9.1	71.9 ± 4.2	72.1 ± 8.6	65.2 ± 8.5	0.001 § *
Gender % male	65	55	68	71	0.730
Diabetes %	9	5	9	0	0.2321
Hypertension %	33	61	72	61	0.149
BSA m^2	1.89 ± 0.18	1.85 ± 0.23	1.93 ± 0.16	1.93 ± 0.14	0.331
Valve area cm^2/m^2	1.95 ± 0.32	1.58 ± 0.33	0.55 ± 0.18	0.77 ± 0.42	<0.0001* ł § ¶ ‖
Heart rate beats/min	59.4 ± 9.4	65.4 ± 8.7	62.6 ± 16.4	60.8 ± 9.1	0.451
Systolic BP mmHg	133.1 ± 11.1	143.2 ± 20.9	143.3 ± 16.4	132.3 ± 16.9	0.052
Diastolic BP mmHg	72.1 ± 7.1	70.8 ± 8.1	67.6 ± 10.2	72.8 ± 7.5	0.202
Aortic SBP mmHg	129.7± 12.0	140.4±21.5	140.0 ± 16.3	130.1 ± 15.6	0.07
LA vol index ml/m^2	34.5 ± 11.7	34.1 ± 11.0	38.5 ± 11.8	39.0 ± 16.3	0.455

Table 4-1. Participant demographics. Overall ANOVA p-value shown in end column. (*p<0.05 TAV vs CONTROLS, ł p<0.05 BAV vs CONTROLS, § p<0.05 ASC vs CONTROLS, ¶ p<0.05 TAV vs ASC, ‖ p<0.05 BAV vs ASC, $ p<0.05 BAV vs TAV.

4.4.1.1 Central blood and augmentation pressure measured by Vicorder applanation tonometry techniques

Central aortic systolic blood pressure did not reach statistically significant differences between groups but aortic pulse pressure was significantly lower in controls and BAV AS than in TAV AS and ASC (Table 4-3). Augmentation of the systolic wave (augmentation pressure) was also greater in TAV AS and ASC by about 14mmHg compared with controls (p=0.004).

4.4.1.2 Distensibility and compliance

Taken as a whole, compliance and distensibility were lowest in ASC and TAV AS and higher in BAV AS and controls, with statistically significant separation between the compliance of BAV AS and ASC (p=0.01) in the ascending aorta (Table 4-3). In all groups, distensibility was higher in the proximal descending aorta than in the ascending aorta with statistical separation between BAV AS and ASC (ASC having the lowest group value and BAV AS the highest; p=0.04).

4.4.1.3 Pulse wave velocity

PWV was fastest (implying stiffest aorta) in those with TAV AS and ASC, compared with controls and BAV AS who had similar, lower values (p=0.0004, Table 4-3).

	CONTROLS (23)	ASC (20)	TAV (22)	BAV (24)	P value (ANOVA)
Aortic PP mmHg	58 ± 7.8	69.7 ± 18.6	73.3 ± 12.7	57.2 ± 12.0	0.0002 *‖ $
Aortic SBP mmHg	129.7± 12.0	140.4±21.5	140.0 ± 16.3	130.1 ± 15.6	0.07
Augmentation pressure mmHg	11.2 ± 4.3	17.8 ± 9.3	19.1 ± 4.5	16.2 ± 6.9	0.004 §*
Distensibility AA 1/mmHg	1.42 ± 0.73	0.93 ± 0.51	1.09 ± 0.74	1.33 ± 0.98	0.16
Distensibility PDA 1/mmHg	2.04 ± 0.86	1.63 ± 0.56	1.86 ± 0.93	2.48 ± 1.27	0.04 ‖
Compliance AA 1/mmHg	1.04 ± 0.53	0.80 ± 0.43	0.83 ± 0.46	1.41 ± 0.98	0.01 ‖ $
Compliance PDA 1/mmHg	0.86 ± 0.37	0.72 ± 0.21	0.83 ± 0.41	1.14 ± 0.44	0.004 ‖ $
Pulse wave velocity cm/s	13.1 ± 4.1	20.5 ± 8.0	18.2 ± 5.7	14.3 ± 5.9	0.0004 § *‖

Table 4-2. Measures of aortic stiffness. Overall ANOVA p-value shown in end column. (*p<0.05 TAV vs CONTROLS, ł p<0.05 BAV vs CONTROLS, § p<0.05 ASC vs CONTROLS, ¶ p<0.05 TAV vs ASC, ‖ p<0.05 BAV vs ASC, $ p<0.05 BAV vs TAV).

To explore correlations with aortic distensibility in more detail, I performed bivariate correlation analysis to assess the relationship with aortic size and aortic hypertension (measured as aortic systolic pressure and augmentation pressure and pulse wave velocity) (Table 4.3). This confirmed the close relationship of aortic distensibility both with other measures of stiffness and with a larger aortic area.

Correlations of distensibility at the ascending aorta						
	AS		TAV AS		BAV AS	
	r	P value	r	P value	r	P value
STJ area	-0.314	0.04	-0.369	0.090	-0.718	0.0002
AA area	-0.425	0.006	-0.377	0.084	-0.413	0.056
Aortic P	-0.546	0.0001	-0.635	0.001	-0.442	0.039
Aug p	-0.538	0.0003	0.720	0.002	-0.425	0.079
PWV	-0.425	0.006	-0.475	0.026	-0.23	0.344

Table 4-3. Correlations of distensibility at the level of the mid ascending aorta.

To explore the confounder of age, an analysis of co variance to adjust for age was repeated with results shown in Table 4-4. PWV remained significantly higher in ASC, after adjustment.

Analysis of covariance between controls and ASC with age adjustment

	Controls	Adjusted Controls	ASC	Adjusted ASC	P value (adjusted means)
Aortic PP mmHg	58 ± 7.8	59.89 ± 3.64	69.7 ± 18.6	67.95 ± 3.53	0.146
Aortic SBP mmHg	129.7± 12.0	131.49 ± 4.47	140.4 ± 21.5	138.70 ± 4.33	0.286
Augmentation pressure mmHg	11.2 ± 4.3	12.79 ± 1.80	17.8 ± 9.3	16.67 ± 1.74	0.156
Distensibility AA 1/mmHg	1.42 ± 0.73	1.29 ± 0.13	0.93 ± 0.51	1.08 ± 0.15	0.328
Distensibility PDA 1/mmHg	2.04 ± 0.86	1.82 ± 0.14	1.63 ± 0.56	1.88 ± 0.15	0.805
Compliance AA 1/mmHg	1.04 ± 0.53	0.95 ± 0.10	0.80 ± 0.43	0.90 ± 0.11	0.762
Compliance PDA 1/mmHg	0.86 ± 0.37	0.77 ± 0.06	0.72 ± 0.21	0.82 ± 0.06	0.604
Pulse wave velocity cm/s	13.10 ± 4.1	14.39 ± 1.28	20.51 ± 8.0	19.04 ± 1.42	0.028

Table 4-4. Adjusted analysis covariance for age between aortic sclerosis and controls. Values presented for adjusted data are mean ± standard error of the mean.

4.4.2 Afterload (2): aortic dimensions and turbulent kinetic energy

The bicuspid aorta was larger at all measures of ascending aortic area (p=<0.0001 between BAV AS and all groups) (Table 4-5). There was no difference in the proximal descending aorta between any group.

Turbulent kinetic energy was highest in the BAV AS group with complete statistical separation of groups from lowest turbulent kinetic energy in controls, to ASC, to TAV AS to BAV AS ($p<0.0001$).

Flow angle, measured at the level of the mid ascending aorta, was more abnormal in the TAV and BAV AS groups compared with controls ($p=0.0003$). ASC had a more abnormal angle than controls, sitting between controls and AS groups, but not reaching statistical significance in comparison with either. There was no difference in the flow angle between TAV and BAV AS (both 24-25°; $p=1.00$). The magnitude vector of in plane and through plane WSS was higher in AS than in either controls or ASC ($p=0.0001$).

	CONTROLS	ASC	TAV	BAV	P value (ANOVA)
STJ area index cm^2/m^2	3.69 ± 0.59	4.13 ± 0.75	3.71 ± 0.77	4.96 ± 1.50	<0.0001 $ ‖ ł
AA area index cm^2/m^2	4.22 ± 0.75	4.94 ± 0.91	4.61 ± 1.20	6.34 ± 1.63	<0.0001 $ ‖ ł
PDA area index cm^2/m^2	2.52 ± 0.50	2.70 ± 0.43	2.59 ± 0.43	2.92 ± 0.60	0.07
TKE mJ	3.89 ± 0.85	6.62 ± 3.23	20.0 ± 8.1	28.8 ± 12.0	<0.0001* ł ¶ ‖ $ §
Flow angle degree	12.4 ± 8.0	18.5 ± 9.3	24.9 ± 11.5	24.4 ± 11.1	0.0003 ł *
Visually eccentric flow %	36	56	75	84	0.03 ł
Mean WSS mag N/m^2	0.47 ± 0.22	0.41 ± 0.14	0.70 ± 0.18	0.63 ± 0.25	0.0001 * ł ¶ ‖

Table 4-5. Aortic dimensions, flow and turbulent kinetic energy. Overall ANOVA p-value shown in end column. (*p<0.05 TAV vs CONTROLS, ł p<0.05 BAV vs CONTROLS, § p<0.05 ASC vs CONTROLS, ¶ p<0.05 TAV vs ASC, ‖ p<0.05 BAV vs ASC, $ p<0.05 BAV vs TAV).

4.4.3 Correlations with turbulent kinetic energy

Peak systolic turbulent kinetic energy increased across groups from controls (lowest) to BAV AS (highest). Significant differences were seen between controls and ASC and between TAV and BAV AS (controls vs. ASC, p=0.006; BAV vs. TAV AS, p=0.03) (Table 4.4). Peak turbulent kinetic energy correlated strongly with valve area in AS (r=-0.583; p=0.0003) (Table 4.4). This association became stronger if the groups were treated individually (BAV AS, r=-0.864, p<0.0001; TAV AS, r=-0.558, p=0.011). Surprisingly there was no correlation with aortic area.

Correlations of TKE						
	AS		TAV AS		BAV AS	
	r	P value	r	P value	r	P value
AVAi	-0.583	0.0003	-0.558	0.011	-0.864	<0.0001
Distensibility	0.111	0.527	-0.153	0.519	0.375	0.168
STJ area	0.211	0.231	0.425	0.06	-0.455	0.102
AA area	0.188	0.280	0.328	0.158	-0.214	0.443

Table 4-6. Correlations with peak turbulent kinetic energy.

4.4.4 Break down of wall shear stress

Wall shear stress was higher in both BAV and TAV AS. Patterns of in plane WSS (Figures 1-3) show that WSS was higher in the anterior portion of the aorta in BAV and generally more evenly distributed/ slightly more left anterior pattern in TAV AS in both magnitude and through plane directions. In plane WSS was non-significantly lower and more evenly distributed in BAV AS. There was no difference between controls and ASC but in plane patterns suggested a more disordered flow with slight flow reversal in the posterior aorta in ASC.

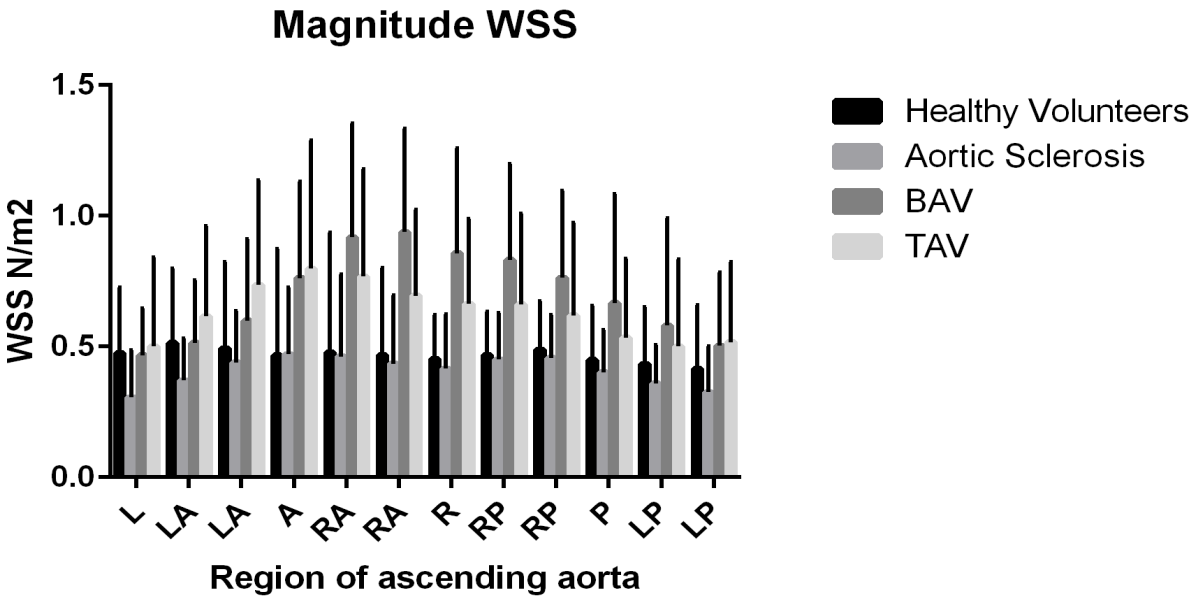


Figure 4-3. WSS (magnitude) across 12 aortic segments (see Fig 4-2 for segmentation). L=left, R=right, A=anterior, P=posterior.

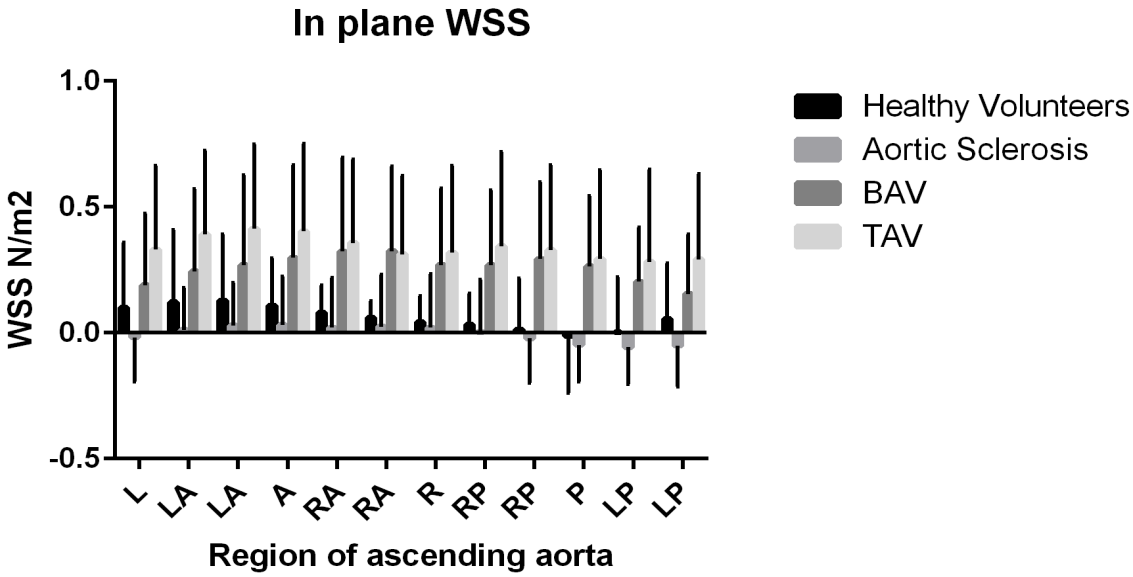


Figure 4-4. WSS (in plane) across 12 aortic segments (see Fig 4-2 for segmentation). L=left, R=right, A=anterior, P=posterior.

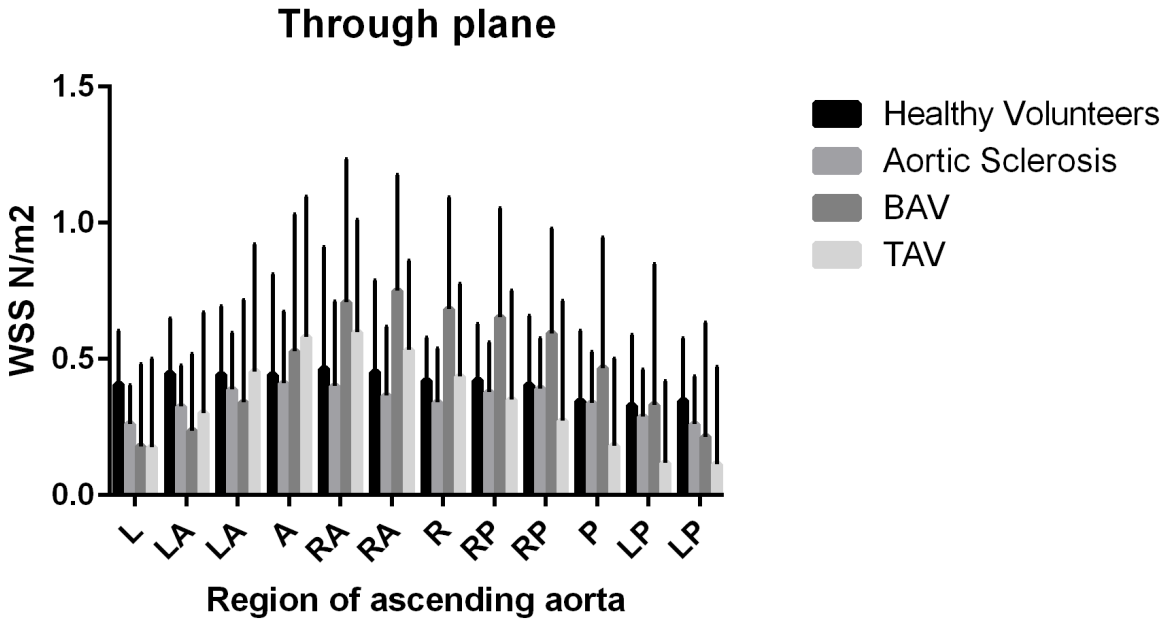


Figure 4-5. WSS (through plane) across 12 aortic segments (see Fig 4-2 for segmentation). L=left, R=right, A=anterior, P=posterior.

4.5.1 Summary of results

Overall measures of aortic stiffness are greatest in those with TAV AS and ASC, in keeping with the older age of participants and the abnormal vascular profile of ASC. There were no important differences between ASC and TAV AS, as might be expected if TAV AS were simply a progression of ASC. This may suggest that ASC and TAV share a common vascular pathology with a small proportion going on to express additional valvular pathology. When compared with controls, ASC is associated both with a stiff aorta and increased turbulent kinetic energy formation.

Compliance is lower in TAV compared with BAV AS with distensibility similar between groups at ascending aortic level although BAV AS have improved distensibility in the descending aorta (beyond the zone of BAV aortopathy, which is confined to the region of insertion of the patent ductus arteriosus) suggesting localised ascending aortic pathology and distensibility, compared with diffuse vascular stiffness in TAV AS. As hypothesised, peak systolic turbulent kinetic energy is higher in BAV AS than TAV AS (despite slightly more severe valve disease in TAV AS) and this observation is not explained by a larger aorta alone.

4.5.2 Advanced measures of blood pressure and aortic stiffness

As well as central blood pressure, the augmented blood pressure is a marker of arterial stiffness, measuring the reflected pressure wave and its contribution to systolic pressure.[158] My results showed that both central blood pressure and augmented pressure

were higher in those with TAV AS and ASC than controls. In an attempt to control for age, the analysis was repeated between controls and ASC. PWV remained significantly worse in those with ASC in the adjusted model. The effect of the older population (6.7-7.9 years) may be a confounder; however, the mean difference in central systolic aortic pressure is large (minimum mean difference of ~ 10mmHg) between the groups, which is more than might be expected for age alone. In a review of the available literature, a large Japanese population study suggests that normal central systolic pressure is between 119-132mmHg in populations without hypertension and around 140mmHg (with standard deviations of up to 37mmHg) in those with known hypertension.[159] Similar findings were shown in an analysis of population surveys and clinical trials, which suggested values of 116-118mmHg in a healthy non-hypertensive population over 70 years old, rising to 135-138mmHg in a population with hypertension or known cardiovascular risk factors.[160] This suggests that the difference observed in the present study is unlikely to be solely due to age and that cardiovascular risk factors are likely to play a contributory role, mirrored by the impaired PWV between TAV AS and ASC (no significant difference) and BAV or controls (p=0.0004).

Compliance is defined as the absolute change in area for a given pressure and distensibility is relative compliance (or relative change in area) for a given pressure. CMR measures have the advantage of providing local information in a well-defined region - in this case, the proximal ascending and descending aorta at the level of the pulmonary artery. Distensibility of the proximal ascending aorta is greatest in controls, and progressively less in subjects with BAV AS and TAV AS, with the greatest impairment in ASC (p=0.01). At the level of the proximal descending aorta, subjects with BAV AS had the highest proportionate improvement and greatest distensibility, followed by controls, then

subjects with TAV AS and ASC (p=0.04 for group driven by BAV AS and ASC), suggesting that subjects with BAV had disproportionate local impairment of distensibility at the level of the ascending aorta, in keeping with existing knowledge.[67] Distensibility was very low across all groups in contrast with many published papers, mainly using younger participants.[85] However, a recently published paper describes changes in aortic distensibility with ageing, confirming that distensibility drops below 2mmHg^{-1} and falls very rapidly beyond the age of 60 years,[86] and agreeing with my findings in controls and that TAV and ASC have lower than expected distensibility but controls also have lower distensibility than was recognised, prior to the publication of this paper.

When measures of aortic stiffness are taken together, the profile was worse in TAV AS and ASC but not different between these two groups. If ASC was simply an early form of TAV AS, the process of vascular impairment may be expected to be worse in subjects with TAV AS and established valve disease. The fact that subjects with ASC had similar (and in many cases slightly worse) measures of vascular stiffness implies a vascular phenotype. Age matched subjects with BAV AS and controls showed very little differences.

4.5.3 Aortic dimensions and peak systolic turbulent kinetic energy

Participants with BAV AS had larger ascending aortas than the other groups (p=<0.0001). No other important group differences in size exist. When compared against subjects with TAV AS, they had significantly higher formation of peak turbulent kinetic energy (28.8mJ compared with 20.0mJ in TAV; p=0.03) despite slightly less severe valve stenosis. My hypothesis suggested that this was due to a larger aortic area; however, peak turbulent kinetic energy did not correlate with mid ascending aortic area nor surprisingly, with the

area at the sinotubular junction, suggesting that other more complex factors are at play and that ELI may not be capturing the same effect. It may be that abnormal flow patterns are implicated and this merits further study.

More predictably, peak turbulent kinetic energy strongly correlated with indexed valve area ($p<0.001$). Peak turbulent kinetic energy measurement has the attractive advantage of remaining independent of mathematical assumptions concerning vessel area from echocardiography. However, given my findings, more work is needed to perform large-scale correlations with echocardiographic and catheter based data before it can be used to accurately estimate pressure loss.[72] My data concerning mean and range of mean turbulent kinetic energy are in keeping with those of Dyverfeldt *et al*, who developed the turbulent kinetic energy sequence and demonstrated wide variation (3-52mJ) in a group of 14 participants with AS of mixed types and severity.[72]

I reported peak turbulent kinetic energy values in ASC for the first time and demonstrated an increase compared with controls (6.62 versus 3.89mJ; $p=0.006$). While this change is small compared with established AS, this equates to irreversible pressure loss with every cardiac cycle, which may impact on LV function over time, especially with the additional load of aortic stiffness. The only available published data for peak turbulent kinetic energy in healthy volunteers showed a peak turbulent kinetic energy of about 3mJ (n=4), in keeping with our data in a larger and older population (mean peak turbulent kinetic energy in controls 3.89 ± 0.85mJ).[72]

4.5.4 Flow angle and WSS

Systolic flow angle was measured with reference to the centre line of flow, which may be measured at any chosen point in the aorta. Previous work has used systolic flow angle in the ascending aorta at the level of the pulmonary artery, which was re-created in this work. I found a very similar degree of flow angulation in BAV AS to that published using the same methodology by Bissell *et al*, who described a mean systolic flow angle of around 30° in patients with BAV AS.[15] In a younger population of healthy volunteers, the mean systolic flow angle was 7.0 ± 4.6°, lower than the mean of 12° in our older population. The other large scale publication using the same sequence and processing techniques in healthy volunteers demonstrated flow angles in the range of 8.4 ± 4.8° at the level of the mid ascending aorta.[20] Whilst normal flow angles have not been systematically studied outside the field of 4D and BAV disease and the effects of aging are unknown, it seems possible that age-related changes to aortic wall function and altered dynamics secondary to aortic and valvular endothelial stiffness may alter flow patterns and flow angle in an older population.

Flow was surprisingly angulated in subjects with TAV AS with no observed difference between TAV and BAV. The implication of these results is surprising. Flow angle was expected to be more abnormal in those with BAV due to the abnormal helicity of flow and the fact that most abnormal flow patterns correlate with more extreme flow angles.[15] However, this is the first piece of work to compare diseased TAV AS with BAV AS and the finding that flow was equally abnormal in TAV AS is novel and of interest. Considering the potential for abnormal valve orifices (the site of initiation of abnormal flow), subjects with TAV AS may demonstrate irregular and non-physiological valve

orifices. However, the normal aortic root size would be expected to potentiate these patterns to a lesser degree than in those with BAV. The concept that TAV AS is associated with abnormal flow could be further explored by analysis of flow patterns.

4.5.5 Visual inspection and grading of flow

Normal flow patterns in the ascending aortic are now well described using 4D flow. Normal flow is usually central or mildly right handed.[15, 20, 161] Abnormal flow is defined as marked right handed, left handed or disordered and is unusual with healthy valves and normal aortic root size. The majority of subjects with ASC had normal flow; however a few had visibly disordered flow.

At the level of the mid ascending aorta, visual assessment of eccentric flow was seen in 36% of controls, 56% ASC, 75% TAV AS, 84% BAV AS. Although these findings corresponded with the flow angle data, this is a crude visual measure only and whilst the observations are interesting, they should be interpreted with caution. A more useful visual assessment of flow is derived from streamline patterns visualised throughout the aorta across the cardiac cycle. Streamline drop out was an unanticipated problem in participants with higher velocities through the valve, requiring a higher VENC with subsequent loss of the lower velocity signals beyond the valve. Older participants also seemed to have poorer image quality and noisier data. Anecdotally, the sequences took longer to acquire in these individuals and it is possible that a pattern of chest wall breathing means the diaphragm navigator is less effective. Personal correspondence with other 4D flow units suggests similar experiences and trials using intravascular contrasts agents indicate that an improved signal to noise ratio is obtained. A new development

(published in abstract form only) is the “circulation tool”, which quantitates the helicity of flow and describes the integral of velocity with respect to aortic cross sectional area, but this methodology was not fully developed for use on the retrospective 4D sequence at the time of this thesis.[162]

4.5.6 Regional WSS

Abnormal flow patterns create a change in the frictional force of blood against the aortic wall in circumferential, axial or vectorial planes (so called regional WSS). Considerable interest has focused on this entity in BAV AS with strong implications that high regional WSS causes the release of matrix metalloproteinases and transforming growth factors, which in turn cause extracellular matrix dysregulation and aortic dilatation.[8, 25, 163] Recent histological studies have further linked regional WSS with regional extracellular matrix dysregulation (decreased elastin and thinner, more dispersed elastin fibres).[26] Despite these well-described changes in BAV, no comparator has examined WSS in TAV AS to date.

At their most simplistic, my results show that mean magnitude WSS was significantly higher in both TAV and BAV AS (compared with controls and ASC) but with no overall difference between subjects with TAV and BAV AS. However, the results become more interesting when broken down in to regional WSS (charts 1-3). WSS mag was higher in the right and anterior portions of the aorta than TAV, despite no overall change in mean value. This pattern is also seen in the through plane WSS. In plane WSS was higher in subjects with TAV and BAV AS than controls and ASC, with no other significant differences. This finding of maximal stress on the anterior aorta in BAV is consistent with

previously published data and histological specimens[15, 26] and in keeping with the described aortopathy.[164]

In summary, there were no differences in mean WSS between subjects with TAV and BAV AS, but the site of maximal regional strain appears to be more anterior in BAV AS. Significant variation is seen within all groups (as demonstrated by the wide standard deviations) but these are nonetheless broadly in keeping with our own published data and that from other units.[15, 20, 26]

4.6 LIMITATIONS

A recognised limitation of WSS is that the resolution of CMR means WSS is a relatively crude indicator of stress near (but not at) the vessel wall. We have measured only systolic WSS whereas diastolic WSS is also implicated in vascular remodelling.[165] Recently described WSS mapping, which can be combined with models of aortic pressure maps, provides a more comprehensive overview of WSS 'hot spots' throughout the chosen region of interest and may be more frequently used in the future.[26] Notwithstanding the current limitations of WSS (including scanning time constraints and very complex analysis), it can provide interesting differences in the patterns of WSS, even if confidence in the absolute values is still unproven.

Small numbers limited the study and an age difference of up to 8 years was present between those in the control group and those with BAV AS compared with those with TAV AS and ASC.

4.7 CONCLUSIONS

Subjects with ASC have a vascular stiffness profile similar to those with TAV AS, in keeping with the hypothesis that ASC represents an adverse vascular phenotype, not simply a manifestation of early valvular dysfunction.

There was clear separation of TKE values in the ascending aorta between all groups. Subjects with ASC have a markedly higher peak turbulent kinetic energy than controls, despite only minimal valve thickening. BAV AS have the highest TKE values, correlation analysis suggests that there may be more to the higher turbulent kinetic energy than simply aortic size in BAV AS.

Flow patterns and WSS have not been previously described in TAV AS. Subjects with TAV AS also have marked flow abnormalities comparable with those seen in BAV AS. Objective measures such as mean WSS are not different in subjects with BAV and TAV AS, although those with BAV AS seem to have more significantly raised WSS in the anterior wall.

In the next chapter, LV geometry, mass and function will be compared across groups to determine if there are insights into LV modelling in the different forms of AVC.

5 LEFT VENTRICULAR RESPONSE: GEOMETRY, MASS AND FUNCTION

5.1 ABSTRACT

5.1.1 Background and aims

Chapter 4 demonstrated that ASC creates afterload as a result of a stiff aorta and increased turbulent kinetic energy compared with controls. Chapter 3 OxVALVE-PCS data showed that participants with ASC had some LV remodelling with a slightly thicker inter ventricular septum and larger LV end diastolic dimension. From these findings, I hypothesised that more sensitive markers of LV dysfunction, such as longitudinal strain impairment were more likely to be impaired in this group, compared with control volunteers. Chapter 4 demonstrated differences in aortic dimension and turbulent kinetic energy between BAV and TAV AS. I hypothesised that strain is more impaired in the BAV AS group as a result of the additional LV load arising from irreversible pressure loss. Aortic valve area and LV mass do not correlate tightly and I explored whether markers of post-valvular afterload correlate better with LV mass.

5.1.2 Methods

89 participants (23 controls, 20 ASC, 22 TAV AS, 24 BAV AS) underwent CMR scanning of LV volumes, mass and function. Feature tracking CMR was used to assess strain and aortic parameters correlated with mass index.

5.1.3 Results

Participants with AS had a higher LV mass than controls or those with ASC (up to 7.8g/m^2, p<0.001). No other significant differences in LV geometry or function were found. Valve area and turbulent kinetic energy correlated with LV mass in AS (r=-0.40, p 0.006 for mass; r=0.53, p=0.001 for TKE), particularly in the BAV AS cohort. Turbulent kinetic energy also showed moderate correlation with LVEDVi (r=0.49, p=0.003), SVi (r=0.56, p<0.001), longitudinal strain (r=0.42, p=0.01) and stroke work index (r=0.58, p<0.001). Correlation with LV mass index was strong in the BAV AS sub group (r=0.83, p=<0.001). TKE independently predicted stroke work in AS, especially in BAV AS.

5.1.4 Conclusions

There were no geometric or functional differences in the LV between groups. Aortic valve area correlated moderately with LV mass index only whereas TKE demonstrated a moderate relationship with LV mass, longitudinal strain, stroke work and LV size, particularly in subjects with BAV AS (a very strong correlation with LV mass index was demonstrated), and may be a promising additional imaging biomarker (beyond valve area alone) in the prediction of LV mass.

5.2 INTRODUCTION

Clinical guidelines place a heavy emphasis on valve area, velocity and gradient when assessing the severity of AS.[101, 166] However, the key determinant of survival is the response of the LV to pressure overload and increasing research interest has centred on this since the advent of sophisticated echocardiography and CMR techniques.[74, 80] While initially compensatory, excess LVH (and thus mass) is detrimental, ultimately causing diastolic dysfunction, myocardial fibrosis and an adverse prognosis (even after valve replacement).[79, 167, 168] An unexpected insight from CMR assessment of moderate to severe AS has been the modest correlation of valve area with the degree of LVH and mass.[36] While there are influences of age, genetics and gender, it is likely that other haemodynamic factors beyond the valve (afterload) influence remodelling.[77]

To summarize in brief, there are two main modifications to standard echocardiographic assessments of AS that are generally accepted, although not in widespread use. Bahlmann *et al* described a modified Bernoulli equation to account for pressure recovery in the smaller aortic root, to improve risk stratification in patients with AS.[70, 71] This was validated in a large clinical study predicting adverse cardiovascular events. The same group then evaluated a further adjustment - the total systemic afterload - in which Briand *et al* summated systolic arterial pressure and mean valve gradient in AS.[56, 69] Both studies used transthoracic echocardiography, which remains the most useful and widely available tool for monitoring of AS, but whose ability to accurately estimate LV mass is inferior to "gold standard" CMR.[169] Echocardiography also relies on assumptions, including extrapolating areas from two-dimensional imaging. Calculations to account for the

energy loss index also rely on assumptions of trans-aortic flow volume, which are based on body surface area. While this technique is useful in large populations, CMR has clear advantages as a research tool, offering excellent visualisation of the heart without the limitations imposed by echocardiographic windows. Views in any LV plane allow accurate calculation of mass and function. Cardiac deformation can be measured as strain at clearly defined and reproducible regions.[170] The exciting ability to measure aortic turbulent kinetic energy adds a new dimension to research concerning energy loss in AS.

In this chapter, I pursued the findings of aortic stiffness and turbulence in the proximal aorta described in the preceding chapter and assess effects on LV geometry, mass and function, incorporating tissue tracking and derived strain, which provides an earlier and more subtle marker of LV deterioration in studies using echocardiographic and CMR strain (tagging).[89] [171] [172] CMR based feature tracking is a newer technique without outcome data in AS. However, measures of global and longitudinal strain show good reproducibility but are higher values are seen in feature tracking, when compared with tagging in AS.[92]

I hypothesised that participants with BAV AS will have higher LV mass and worse strain profile than TAV AS (despite younger age) because energy 'losses' (turbulent kinetic energy conversion to heat) are higher. As demonstrated in Chapter 3, participants with ASC are also likely to have early adverse remodelling, possibly as a result of increasing afterload secondary to vascular stiffness and turbulence.

5.3 METHODS

A total of 89 participants (23 controls, 20 ASC, 22 TAV AS, 24 BAV AS) gave informed consent to CMR scanning, as described in Chapter 2. Cardiac volumes, mass and ejection fraction were acquired from SSFP cines as previously described in Chapter 2.

Stroke work is the LV work required to eject blood into the aorta and is the product of stroke volume and mean arterial pressure:

$$Stroke\ work\ (SW)$$
$$= Stroke\ volume\ (SV)mls \times mean\ arterial\ pressure\ (MAP)mmHg$$

Increased sphericity is a marker of adverse remodelling in heart failure[173] and calculated as:

$$Sphericity\ =\ LV\ long\ axis\ length\ \div\ LV\ short\ axis\ length$$

Myocardial strain is the relative deformation of the myocardium during contraction and relaxation.[174] Strain is reported as a percentage change of myocardial deformation, referring to the change of myocardial fibre length at end-systole compared to its original length at end-diastole. Strain rate is the change in strain per unit time.[175] With contraction, the myocardium is shortened (negative strain) but lengthens during relaxation (positive strain). Strain can be measured in longitudinal, circumferential and radial directions and is thought to partially reflect sub-endocardial, mid-wall and sub-epicardial myocardial function, respectively.

Myocardial strain was measured using feature tracking (CMR-FT) TomTec analysis software (TomTec, Unterchleissheim, Germany) as previously described in main methods. The basal slice was defined as the most basal short axis slice with a complete rim of myocardial tissue seen throughout the cardiac cycle. The apical slice was defined as the last slice with a complete rim of myocardium. The mid LV slice was taken as the midpoint between the basal and apical slices.

Outcome parameters measured in this study were ventricular peak systolic circumferential strain and strain rate, peak global systolic longitudinal strain and strain rate. Best validated in the literature and quoted in this thesis are circumferential and longitudinal strain. Early diastolic strain rate (EDSR) was measured to reflect diastolic function.

5.4 RESULTS

The mass index was higher in the AS groups than in controls or those with ASC ($p<0.001$). Otherwise, no significant differences existed in LV geometry or function between groups (Table 5-1). LV EF was normal across all groups. Left atrial (LA) maximal area was larger in the AS groups than in controls and ASC but this difference was not statistically significant. Strain did not differ amongst groups with values lying within the published normal range (Table 5-2).[176] Participants with BAV AS had consistently lower strain values than those with TAV AS (p=NS).

	CONTROLS (23)	ASC (20)	TAV (22)	BAV (24)	P value (ANOVA)
Age (years)	64.2 ± 9.1	71.9 ± 4.2	72.1 ± 8.6	65.2 ± 8.5	0.001 § *
Gender %Male	65	55	68	71	0.730
BSA m2	1.89 ± 0.18	1.85 ± 0.23	1.93 ± 0.16	1.93 ±0.14	0.331
Valve area cm2/m2	1.95 ± 0.32	1.58 ± 0.33	0.55 ± 0.18	0.77 ± 0.42	<0.0001* ł § ¶ ‖
Heart rate beats/min	59.4 ± 9.4	65.4 ± 8.7	62.6 v 16.4	60.8 ± 9.1	0.451
Systolic BP mmHg	133.1 ± 11.1	143.2 ± 20.9	143.3 ± 16.4	132.3 ± 16.9	0.052
Diastolic BP mmHg	72.1 ± 7.1	70.8 ± 8.1	67.6 ± 10.2	72.8 ± 7.5	0.202
LVEDVi ml/m^2	73.9 ± 14.9	71.4 ± 13.5	72.2 ± 12.6	81.5 ± 18.5	0.101
LVESVi ml/m^2	25.1 ± 6.7	23.1 ±7.3	22.1 ± 7.8	26.4 ± 11.1	0.326
SVi ml/m^2	49.3 ± 10.1	48.2 ± 8.6	50.0 ± 6.3	54.8 ± 12.4	0.122
Sphericity	1.86 ± 0.19	1.82 ± 0.18	1.81 ± 0.21	1.76 ± 0.35	0.569
Stroke work (SV x MAP)	0.96 ± 0.27	0.92 ± 0.26	1.01 ± 0.30	1.14 ± 0.39	0.161
Mass index g/m^2	60.3 ± 10.3	62.1 ± 14.8	77.1 ± 13.4	78.1 ± 22.2	0.0001 ł * ¶ ‖
LVEF %	66.3 ± 5.3	68.1 ± 6.7	71.4 ± 6.1	68.2 ± 8.7	0.133
LA max area cm^2	21.8 ± 5.1	21.8 ± 4.1	24.2 ± 5.1	24.4 ± 6.6	0.205

Table 5-1. Demographics, LV volumes, mass and function. Overall ANOVA p-value shown in end column. (*p<0.05 TAV vs CONTROLS, ł p<0.05 BAV vs CONTROLS, § p<0.05 ASC vs CONTROLS, ¶ p<0.05 TAV vs ASC, ‖ p<0.05 BAV vs ASC, $ p<0.05 BAV vs TAV).

	CONTROLS (23)	ASC (20)	TAV (22)	BAV (24)	P value (ANOVA)
Longitudinal Strain Parameters					
Strain	-19.2 ± 5.3	-20.2 ± 7.1	-20.5 ± 4.4	-17.6 ± 4.3	0.339
Strain rate	-1.1 ± 0.4	-1.3 ± 0.5	-1.3 ± 0.4	-1.0 ± 0.3	0.082
Early Diastolic SR	0.9 ± 0.3	1.0 ± 0.5	1.0 ± 0.3	0.9 ± 0.3	0.715
Circumferential Strain Parameters					
Basal strain	-24.7 ± 4.0	-25.4 ± 4.7	-26.9 ± 4.5	-24.1 ± 4.6	0.269
Basal SR	-1.4 ± 0.3	-1.5 ± 0.4	-1.5 ± 0.4	-1.3 ± 0.3	0.299
Mid strain	-22.7 ± 4.8	-23.8 ± 6.2	-26.0 ± 5.6	-23.2 ± 7.0	0.320
Mid SR	-1.3 ± 0.5	-1.4 ± 0.5	-1.6 ± 0.4	-1.4 ± 0.5	0.346
Apical Strain	-35.4 ± 9.2	-32.3 ± 12.2	-35.8 ± 11.2	-31.7 ± 10.5	0.502
Apical SR	-2.7 ± 0.8	-2.7 ± 1.5	-3.1 ± 1.5	-2.4 ± 1.2	0.622
LA vol index ml/m^2	34.5 ± 11.7	34.1 ± 11.0	38.5 ± 11.8	39.0 ± 16.3	0.455

Table 5-2. Results of peak systolic strain and strain rate (SR). Overall ANOVA p-value shown in end column. (*p<0.05 TAV vs CONTROLS, ł p<0.05 BAV vs CONTROLS, § p<0.05 ASC vs CONTROLS, ¶ p<0.05 TAV vs ASC, ǁ p<0.05 BAV vs ASC, $ p<0.05 BAV vs TAV).

To test the relationship between valve area and mass index, bivariate correlation was performed (Table 5-3). In the AS group as a whole there was a moderate correlation (r = -0.404; p=0.006). In individual group analysis a moderate correlation was found in participants with TAV (r= -0.381; p=0.08) and a stronger correlation in those with BAV (r= -0.466; p= 0.03).

Further bivariate correlation was undertaken to establish relationship between mass and other parameters relating to aortic function (Table 5-3). No correlation was found with aortic distensibility, pulse wave velocity or augmentation pressure. Mass correlated moderately with longitudinal strain rate (r=0.351; p= 0.026) and stroke work (r=0.459; p= 0.003), and strongly with turbulent kinetic energy (r= 0.53; p= 0.001).

Correlations with mass index						
	AS		**TAV AS**		**BAV AS**	
	r	**p**	**r**	**p**	**r**	**p**
AVAi	-0.404	0.006	-0.381	0.08	-0.466	0.029
Aortic Distensibility	0.105	0.503	0.303	0.182	0.185	0.409
PWV	0.008	0.962	0.081	0.72	0.097	0.691
Aug pressure	-0.133	0.408	-0.400	0.065	0.039	0.873
TKE	0.530	0.001	0.203	0.391	0.830	0.0001

Table 5-3. Correlations with mass index.

Correlations of LV parameters with peak turbulent kinetic energy were assessed and demonstrated moderate or strong correlations with LV size, stroke volume and stroke work and longitudinal strain, especially in BAV AS. No correlation was found with LV function as measured by ejection fraction or longitudinal strain. Sphericity of LV was not correlated with peak turbulent kinetic energy.

Correlations with TKE						
	AS		TAV AS		BAV AS	
	r	p	r	p	r	p
LVEDVi	0.486	0.003	-0.126	0.5976	0.688	0.005
SVi	0.564	0.0004	-.044	0.853	0.784	0.001
LVEF	-0.087	0.621	0.072	0.762	0.026	0.928
Long strain	0.417	0.013	0.365	0.114	0.661	0.007
Stroke work	0.649	<0.0001	0.363	0.115	0.827	0.0003
Stroke work index	0.58	0.0003	0.21	0.373	0.833	0.0002
LV Sphericity	0.238	0.169	0.16	0.498	0.359	0.189

Table 5-4. Correlations with peak turbulent kinetic energy.

To compare the strength of correlations of LV parameters with peak turbulent kinetic energy against indexed aortic valve area, the analysis was repeated (table 5-5). Turbulent kinetic energy was more strongly correlated for most LV parameters than valve area, except ejection fraction, which correlated weakly with mass. The sphericity of the LV correlated inversely with valve area in the BAV AS cohort, as did longitudinal strain.

In order to assess whether remodelling parameters were predictive of the turbulent kinetic energy, a multiple linear regression model was created. The independent variables entered into the model were LVEDVi, SVi, longitudinal strain rate and SWi. The

independent predictor of the TKE was found to be SWi (β = 0.467, P = 0.0002) overall R^2 of the model = 0.22, P = 0.0002. In order to avoid co-linearity of predictors the other prognostic cardiac remodelling parameter of EF was substituted into the model above instead of LVSVi, but in this subsequent model SWi remained the only independent predictor.

The indexed aortic valve area was log transformed in order to perform an additional multiple linear regression analysis to assess whether remodelling parameters were predictive of the aortic valve area. The independent variables entered into the model were LVEDVi, SVi, longitudinal strain rate and SWi. The independent predictor of the AVAi was found to be SWi (β = -0.288, P = 0.016) overall R^2 of the model = 0.083, P = 0.016.

Correlations with AVAi						
	AS		TAV AS		BAV AS	
	r	p	r	p	r	p
LVEDVi	0.021	0.891	0.171	0.445	-0.164	0.466
SVi	-0.178	0.248	-0.031	0.89	-0.336	0.126
LVEF	-0.332	0.028	-0.235	0.293	-0.301	0.174
Long strain	0.215	0.189	-0.203	0.366	-0.488	0.021
Stroke work	-0.23	0.159	-0.235	0.293	-0.35	0.169
Stroke work index	-0.192	0.241	-0.225	0.314	-0.283	0.27
LV Sphericity	-0.25	0.102	0.083	0.713	-0.434	0.04

Table 5-5. Correlations with indexed aortic valve area

5.5 DISCUSSION

As might be expected, LV mass index is higher in those with AS; an adaptive response to afterload. However, despite significant differences in load between groups, no significant differences in LV geometry or function (including LV strain) were found. This suggests any such changes arise at a very late stage or are too subtle to be detected in small group numbers, despite the sensitivity of CMR. The relationships with LV mass provide new insight into additional loading factors, with peak turbulent kinetic energy showing a strong correlation with LV mass, driven mainly by the BAV AS cohort.

5.5.1 Aortic Sclerosis

In Chapter 3, I observed a clinically small but strongly statistically significant increase in septal thickness and larger LV end diastolic diameter in an unselected and age-matched population with ASC compared with normal controls. Chapter 4 demonstrated greater aortic stiffness and higher peak turbulent kinetic energy formation in ASC than in controls, albeit with the caveat that the control group were younger. These data gave credence to the hypothesis that LV mass index would be higher in ASC and that change in LV geometry and function might be observed. In fact, this has not been borne out. It may be that despite the accuracy of CMR, the sample size is likely to be too small to detect subtle changes. The data from Chapter 3 were derived in much higher numbers, which can demonstrate group differences, especially given the broad range of normal values of LV size and mass within a population. Strain rates were remarkably similar between controls and participants with ASC.

A literature review reveals a paucity of research on LV function in ASC. Reflecting our findings, the only published paper found no relationship with LV mass in a small ASC population compared with controls.[177] There have been no studies of strain abnormalities in ASC.

Similarly, there were no significant differences (other than a higher mass index) between participants with TAV AS and those with ASC, perhaps because the studied TAV AS group remained compensated at the time of study, with potential for strain reduction later in the disease process.

5.5.2 Aortic Stenosis

As expected, LV mass index was significantly higher in those with AS than in controls or ASC (controls 60.3g/m^2; ASC 62.1g/m^2; TAV AS 77.1g/m^2; BAV AS 78.1g/m^2; $p<0.001$) and correlated with valve area ($r=-0.40$; $p=0.006$). This correlation was moderate in TAV ($r=-0.381$; $p= 0.08$) and BAV AS ($r=-0.466$; $p=0.029$). While this correlation is stronger than found by Dweck *et al* in their CMR study of 91 patients with AS (55 BAV), given the association between mass and valve area, it seems likely that there is more to account for the variability in LV mass, providing credence for the notion that remodelling is governed by more than valve area alone.[36] The stronger relationship between valve area and mass in the BAV AS group is interesting and merits further exploration. Furthermore, valve area in BAV AS correlated inversely with sphericity, suggesting it may predict remodelling.

Measures of post valvular afterload (as measured in Chapter 4) may be important in determining increases in LV mass. To test this further, bivariate correlation analysis was performed to assess measures of aortic stiffness and turbulent kinetic energy. In a

combined analysis of AS, none of the markers of aortic stiffness (distensibility, PWV and augmentation index) were correlated with mass index. Turbulent kinetic energy has not previously been correlated against LV mass index and the relationship is interesting. For AS as a whole, the relationship was moderate ($r=0.530$; $p=0.001$) and the group breakdown informative. For TAV AS alone, the relationship was lost ($r=0.203$; $p=0.391$) while the association remained very strong in subjects with BAV AS ($r=0.83$; $p<0.0001$), suggesting that turbulent kinetic energy (and thus irreversible pressure loss) has an important positive correlation with LV mass in this cohort. This is the first time that turbulent kinetic energy has been related to LV mass in AS and the strong correlation in subjects with BAV AS suggests that total afterload in this cohort is a component of inefficient energy conservation. Further studies to confirm this relationship may be helpful in answering why BAV AS and valve area are much more tightly related than in TAV AS and whether turbulent kinetic energy is the sole driver. When tested as an independent predictor, stroke work index was strongly predicted by both TKE and aortic area index.

Peak turbulent kinetic energy in the ascending aorta also correlated with LV size and work. These correlates are again particularly strong in BAV AS, giving further credence to the emerging potential of turbulent kinetic energy as a predictor of LV decompensation in BAV. Moreover, the BAV AS group exhibited a non-significant but consistently impaired profile suggesting adverse LV remodelling and functional changes (higher LV mass, larger LV and reduced strain; p= all NS), despite a younger age with non-significantly less severe valve disease. Although no conclusion can be drawn from non-significant results, it is noteworthy that all parameters were “worse” than their TAV AS counterparts, which

could relate either to longer disease duration or more pronounced energy losses (and hence increased LV work). There are no published data comparing LV function in participants with BAV and TAV AS. However, other echocardiographic studies have shown reduced LV strain in non-diseased BAV and healthy volunteers.[178-180] An echocardiographic study of 40 young adults with BAV found larger systolic and diastolic LV dimensions, increased aortic stiffness and reduced longitudinal, circumferential and radial strain when compared with healthy volunteers.[180] A more recent study of 35 young adults with BAV found a lower LV ejection fraction and diastolic abnormalities.[178] Echocardiographic studies in athletes with a normally functioning BAV found reduced basal strain and a further study which monitored athletes with BAV over 5 years found that LV dilatation (although within normal limits) occurred in BAV but not in healthy controls.[179] Finally, a small echocardiographic study of 33 young BAV patients with aortic diameters of <35mm and no significant valve disease demonstrated reduced longitudinal strain but no difference in aortic function (as measured by distensibility, strain and aortic annulus elastic modulus),[181] leading to the hypothesis that BAV might be associated with an inherent cardiomyopathy. However, there is little support for this. Post mortem data have failed to demonstrate myocardial abnormalities and while BAV is associated with a spectrum of other congenital structural abnormalities, it has never been shown to be associated with a cardiomyopathy *per se*.

A more likely alternative hypothesis is that energy losses in the proximal aorta as a result of turbulence and abnormal aortic stiffness (as demonstrated in Chapter 4) lead to greater LV work, LV remodelling and adverse function. However, larger study numbers would be needed to show this more conclusively. In the diseased state, I demonstrated

that while participants with BAV AS have lower blood pressure and reduced global aortic stiffness, they also have regional impairment of aortic distensibility (comparable with TAV) and the additional insult of higher turbulent kinetic energy (and, by association, less pressure recovery). These factors could interact to create increased LV demand and decompensation at an earlier point in the disease pathway. There are some intriguing signals from the LV data, but numbers are too small to draw further conclusions.

CMR-feature tracking (CMR-FT) is a relatively new technique which has been validated against standard CMR tagging and agrees well with circumferential strain (but less so with longitudinal strain).[182] These changes are between group differences and the absolute strain values are not abnormal in themselves when compared with the (limited) published normal values using this technique. Previous publications have shown that global longitudinal strain in lower in subjects with severe AS, and improvement is seen after aortic valve replacement. Circumferential strain is more mixed with some authors showing supranormal (especially mid and apical) strain and other (in the very severe group) showing a drop which improves with aortic valve replacement.[90, 91, 183] However, methods to assess strain differ and studies to date using feature tracking CMR have been small and conducted mainly in healthy volunteers.[182, 184] Publications concerning the use of CMR-FT in AS are few and normal values for LV mass, size and function in larger studies are required for further validation. However, longitudinal strain correlated with turbulent kinetic energy, and to a lesser extent indexed valve area. This suggests that strain is becoming impaired as the LV remodels, although I was unable to detect absolute differences between groups. Once again, this relationship is stronger in BAV AS.

5.5.3 Limitations

This study only examines resting parameters and the ability to increment cardiac output with a fixed obstruction is very relevant in AS. Exercise studies within the confines of a CMR unit are challenging (and accurate LV volumes and 4D flow difficult to acquire due to increased patient movement) and only a few UK centres have this facility.

Small numbers and a previously described age difference between groups are limitation to this study. No tissue characterisation of assessment of occult coronary artery disease was undertaken.

5.6 CONCLUSIONS

Resting LV function in participants with ASC does not differ from controls or from those with established AS. Participants with BAV AS demonstrate a tighter relationship with LV mass and valve area than those with TAV AS. An additional a contributory effect of afterload is suggested by a strong positive correlation with peak turbulent kinetic energy. This association was particularly evident in the BAV group, suggesting that turbulent kinetic energy may play an important role in future valvular research. Longitudinal studies and correlation with clinical outcome would be priorities.

6 CARDIOPULMONARY EXERCISE TESTING AND WHOLE HEART FLOW; A COMPARISON OF ESTABLISHED BICUSPID AND TRICUSPID AORTIC STENOSIS

6.1 ABSTRACT

6.1.1 Introduction

Whole heart 4D flow has shown promise in evaluating flow components and their end diastolic kinetic energy in dilated cardiomyopathy. It's feasibility and utility in AS has not been assessed. In this chapter, I evaluated the use of whole heart 4D flow in AS and controls and compare the physiological output of patients with BAV and TAV AS using CPET.

6.1.2 Methods

12 patients with AS (6 BAV AS, 6 TAV AS) underwent CPET exercise testing and whole heart 4D flow. An age- matched control group of 12 healthy volunteers was used as a comparator for whole heart 4D flow only.

6.1.3 Results

Of 12 AS datasets, 8 were suitable for analysis. The remainder were unsuitable due to excessive ventricular ectopy or the scanner exceeding memory capacity. No difference was shown in the proportion of flow components or end diastolic kinetic energy. Moreover, there was no difference in cardiovascular output between BAV and TAV AS

using CPET based measures of VO_2 max and RER. A slightly lower direct flow component was seen in the combined AS group compared with controls (33.8% compared with 38.8%, p=0.003)

6.1.4 Conclusion

No important differences in flow components of kinetic energy profiles were seen between the small BAV and TAV AS groups. The direct flow component was slightly reduced in AS compared with controls. Acquisition of whole heart 4D flow remains technically challenging. CPET assessment did not reveal differences in exercise capacity between those with BAV and TAV AS.

6.2 INTRODUCTION

The dominant principle of blood flow in the human heart is 'first in - first out' and the creation of helical flow optimises flow from the inflow to the outflow of the LV.[185] Whole heart 4D flow sequences have been developed over the last few years to visualise and quantify blood flow throughout the cardiac cycle. This technique has evolved from the earlier 4D flow sequences which were mainly used to assess flow in vascular structures. The advent of retrospective gating and shortened acquisition times now mean that whole heart 4D flow is a reality, allowing measurement of kinetic energy utilisation and the transport of blood through the LV.[121] While this technique is still in relative infancy, it has shown clear changes in the available end diastolic kinetic energy in systolic heart failure.[96] Moreover, a larger proportion of inflow is retained (and not ejected) during the next immediate beat in dilated cardiomyopathy.[96] Research is on going into flow and energy alterations in heart failure with preserved ejection fraction (which would be most analogous to LV remodelling in AS) but no published work exists.

Flow within the LV is dynamic, with regions of straight and turning flow, flow-flow interactions, recirculating masses of blood, and vortices that develop and extinguish with phases of the cardiac cycle.[121] Eriksson *et al* have described and simplified the four separate components, as follows:[121]

Direct flow: Blood that enters the LV during diastole and leaves the LV during systole in the analysed heart beat.

Retained inflow: Blood that enters the LV during diastole and resides in the LV throughout the cardiac cycle.

Delayed ejection flow: Blood that starts and resides within the LV during the diastolic phase of the analysed cardiac cycle and is ejected during the systolic phase.

Residual volume: Blood that resides in the LV for at least two cardiac cycles and is not a component of inflow or ejected blood.

Visual pathlines are defined as one of the four flow components according to their relative positions at end-diastole and end-systole. These components are illustrated in Figure 6-1.

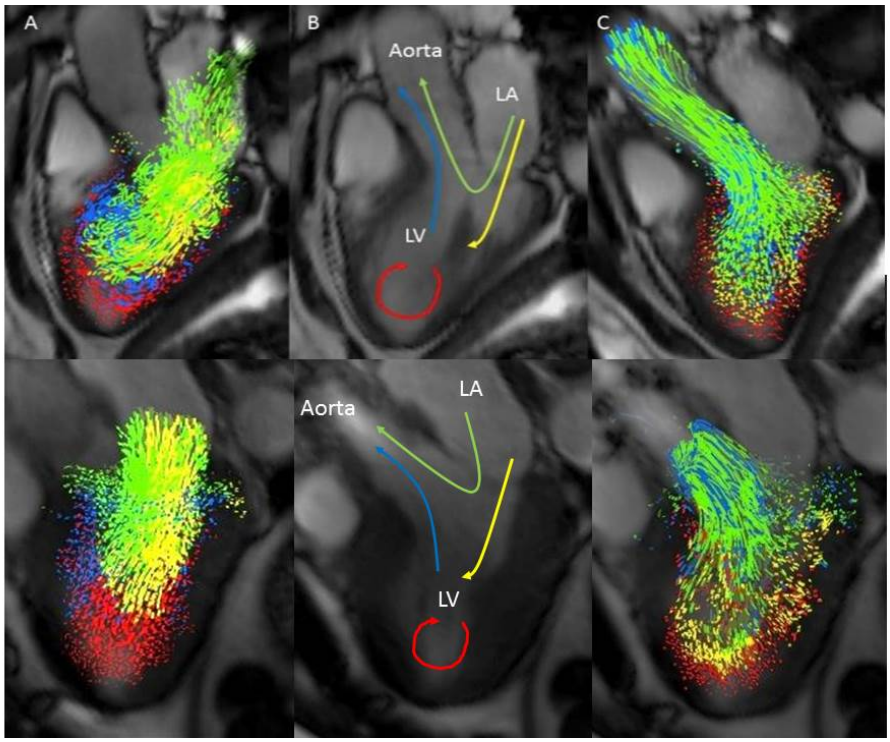


Figure 6-1. Left ventricular flow components. Direct flow, green; retained inflow, yellow; delayed ejection flow, blue and residual volume, red. Panel (A) shows diastolic flow, (B) shows the direction of movement of each flow component and (C) demonstrates systolic flow in a healthy volunteer. The bottom row demonstrates the same time period in a patient with TAV AS. Note the abrupt loss of pathlines just at and above the valve due to aliasing from the low VENC needed in whole heart flow.

As well as visualising and describing flow, whole heart 4D flow can measure the kinetic energy of each component of blood flow at any point of the cardiac cycle. Kinetic energy is defined as the work required to deliver a volume of blood along a chosen pathline (or distance).[123, 186] The CMR sequence calculates KE using an equation that utilises the volume of blood represented by a pathline, the velocity of blood at a chosen time point in that pathline, and blood density. The KE of each pathline within a flow component is then summed to calculate the total KE for that flow component. An example of a kinetic energy profile is shown in Figure 6-2.

Currently unpublished work (abstract data is referenced below) has shown that changes in the component end-diastolic KE profiles in dilated and ischaemic cardiomyopathy relate to exercise capacity, as measured by a standard 6 minute walk distance.[187]

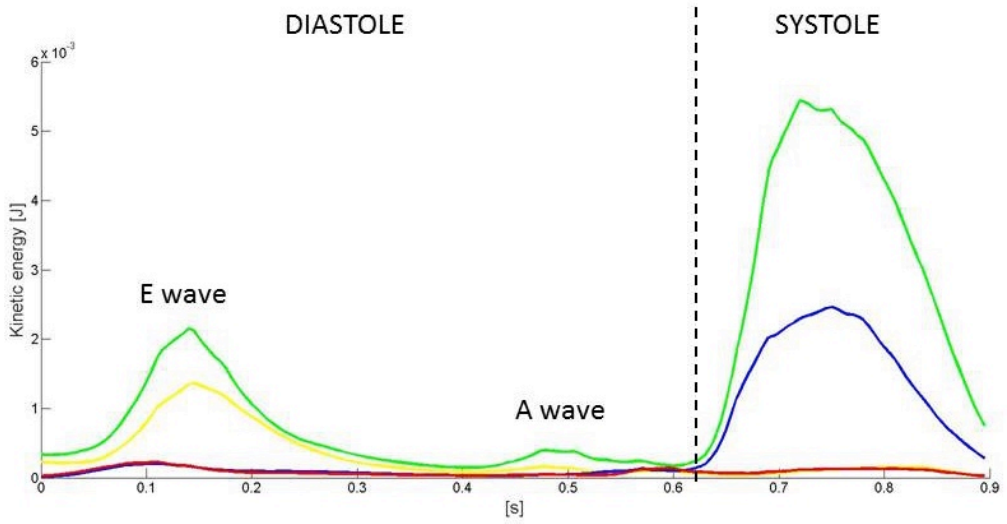


Figure 6-2. Typical kinetic energy profile (in health) for the four flow components. Direct flow, green; retained inflow, yellow; delayed ejection flow, blue and residual volume, red.

In this thesis, we have established adverse kinetic energy conservation in the proximal aorta in BAV compared with TAV AS (via the formation of more turbulent kinetic energy)

but have not clearly shown a difference in LV function between groups. In this final chapter, I describe early data and a description of how CPET and whole heart 4D flow may be used to assess physiological demands.

As previously explored, the complex dynamics of valvular, arterial and ventricular function are key to the development of symptoms in AS. Many participants reduce their activities and do not notice insidious exertional symptoms until a relatively late stage. We know from data concerning European clinical practice that many patients with AS are operated on too late in the disease since symptoms are not recognized as valvular in origin.[188] Cardiovascular "fitness" and physiological demands are individualised and variable (eg. valve area relative to body size, concurrent hypertension or coronary artery disease, and activity levels).[36, 70, 71]

Although symptom onset is crucial to outcome, there are difficulties with objective assessment. While attempts to encourage exercise testing are cemented in guidelines, they are not universal in standard clinical practice,[87, 166, 189] reflecting the increasing age and complex comorbidity in this population, and physician indifference. The advent of minimally invasive aortic valve replacement (transcatheter aortic valve replacement) has expanded the population undergoing evaluation for aortic valve intervention to include an older and frailer subset of patients than was typical even a decade ago. Basic exercise testing is often impractical in this cohort with results affected by the degree of left ventricular hypertrophy (which itself causes ECG abnormalities) and the ability of patients to cope with treadmill exercise.

The argument to support greater use of exercise testing is, however, clear-cut. Exercise testing can unmask symptoms in up to 30% of the supposedly asymptomatic group with AS.[190,191] A positive stress test has important implications for survival; only 20% of those with severe AS and a positive stress test were event free at 2 years, compared with 85% of those with a negative test.[190]

Cardiopulmonary exercise testing has advantages over basic treadmill exercise testing. First, it gives more objective measures of exercise capacity - the most commonly used is peak oxygen consumption (VO_{2max}), which is an independent predictor of prognosis in chronic heart failure.[192] Second, CPET has the advantage that it often uses stationary bicycle exercise, which overcomes many of the problems related to joint loading and balance reported with treadmill exercise. Few studies have been performed using CPET in AS but the available data suggest it is safe.[193, 194] Patients with moderate to severe asymptomatic AS who are limited by symptoms on exercise testing have lower peak VO_2.[194]

The aim of the current study was to assess the feasibility of whole heart 4D flow in participants with AS, to compare with controls and to determine the relationship of flow components and end diastolic kinetic energy with peak VO_2 in patients with BAV and TAV AS.

6.3 METHODS

A sub set of 12 asymptomatic participants who were willing to undergo CPET testing and further 4D CMR flow were recruited from the main study. 12 age matched controls were

used from a previous study, using the same CMR methodology. This control group did not undergo CPET. All exercise tests were physician supervised with indications for termination as previously published.[195]

6.3.1 Cardiopulmonary Exercise Test

Symptom-limited exercise testing was performed on a stationary bicycle ergometer (Ergoline, Bitz, Germany) using a 1-minute ramped protocol with specific workloads, modified by an experienced operator according to age and gender but based on standard protocols.[196, 197] A 12-lead electrocardiogram was monitored continuously and blood pressure recorded every 2 minutes. Expired ventilatory gases were analysed using the Cortex Metalyzer 3B system (Cortex Leipzig, Germany) to determine peak VO_2 alongside the respiratory exchange ratio (RER, a marker of exercise intensity).

6.3.2 Whole Heart 4D Flow

All study participants underwent CMR scanning at 3T for retrospectively ECG gated 4D flow data acquisition and LV anatomical imaging. Whole heart differs from the aortic sequence as it covers a larger area and the voxel size is larger. Data acquisitions were acquired with respiratory navigator gated three-dimensional, three-directional, time resolved phase contrast CMR sequence (3D cine PC-CMR) using a retrospective ECG trigger. The echo time was 2.75ms with a repetition time of 4.3ms and temporal resolution of 52ms. The flip angle was 7°, read field of view 390mm and voxel size 3x3x3mm, as described by Erikkson *et al*.[121] The velocity encoding was 1.0m/s. A sagittal field-of-view (FOV) was adjusted for each participant to encompass the whole heart.

6.3.3 Analysis

Data analysis was performed based on the methods developed by Erikkson *et al*.[121] Velocity data were converted into a file format compatible with commercially available visualisation software (EnSight, CEI Inc., Research Triangle Park, NC, USA). Data sets were analysed using Segment software (Segment, version 1.9 R2842) to manually segment the LV endocardium from short-axis (SA) images at end-diastole and end-systole.[121] The segmentation at end-diastole was re-sampled to provide a volume with isotropic voxels equal to the size of the flow data voxels. A pathline was then emitted from the centre of each voxel included in the LV segmentation and pathlines created forwards and backwards in time until the preceding or subsequent end-systole, respectively. In combination, these forward and backward pathlines represent the entire LV end-diastolic volume tracked over one complete cardiac cycle. The positions of all pathlines at the time of end-systole relative to the LV cavity (as defined by the segmentation at end-systole) were then used to divide them into four pre-specified functional flow components; direct flow, retained inflow, delayed ejection flow and residual volume, as described previously.[121] Kinetic energy for each blood component was recorded at end-diastole, values at this time point reflecting preservation of inflowing kinetic energy prior to the rapid ejection of blood during systole. The kinetic energy values were calculated over the cardiac cycle.

Part of the challenge of using whole heart 4D flow in the sequence is reliant on the avoidance of aliasing within the analysis. The optimal VENC for sensitivity to intra-cardiac flow is around 1.0m/s, which gives a buffer of up to about 1.7m/s. Severe AS can be associated with acceleration of blood flow through the LV outflow tract, which can be

further narrowed in some cases secondary to certain patterns of LVH. However, aliasing was not a problem in the acquired datasets and the segmentation avoided the LVOT itself.

6.4 RESULTS

Acquisition of 4D flow was incomplete or unsuitable for analysis in 4 cases, leaving only 8 complete datasets and 12 CPET datasets. The reasons for 4D flow failure were ectopic beats, patient movement within the scanner, or scanner failure due to image reconstruction limits. All datasets were analysable using the tools developed for healthy volunteers and participants with cardiomyopathy. Aliasing of flow within the LVOT did not occur within the short axis stack.

There were no significant differences in participant age, gender or basic valve and LV parameters. Participants with TAV AS achieved a slightly higher workload (as measured by VO_2) but study numbers were small without statistical power.

	TAV (n = 4)	BAV (n = 4)	P value
Gender % male	83.3%	50.0%	0.221
Age at scan	65.5 ± 10.2	64.2 ± 7.0	0.798
AVA index cm^2/m^2	0.65 ± 0.17	0.82 ± 0.34	0.284
LVEDVi mls/m^2	73.4 ± 12.6	78.1 ± 18.1	0.607
SVi mls/m^2	49.4 ± 6.7	49.6 ± 14.8	0.977
LVEF %	68.4 ± 4.4	70.1 ± 16.2	0.817
LV mass index g/m^2	79.3 ± 14.2	72.7 ± 23.2	0.565
Peak Watts	130.0 ± 40.5	119.2 ± 55.4	0.707
V02 max ml/kg/mn	17.6 ± 5.9	15.5 ± 7.2	0.551
RER peak	1.15 ± 0.1	1.17 ± 0.8	0.818
Rise in SBP mmHg	54.2 ± 10.3	33.3 ± 29.3	0.131

Table 6-1. Participant demographics, LV volumes, function and basic CPET outcome.

There were small differences in the proportions of flow components, with a higher delayed ejection flow of blood in participants with TAV AS. These were slightly higher than would be expected from published normal data (16 ± 3%); though it is unclear whether this is a true disease-specific pattern or a consequence of the small study numbers and large standard deviation in this particular group. Due to the same numbers, the AS was then combined and compared against age and gender matched controls (Table 6-3).

	TAV	BAV	P value
Flow components %			
Residual volume	27.8 ± 7.9	32.6 ± 1.2	0.06
Delayed ejection flow	21.8 ± 8.0	18.1 ± 4.3	0.048
Retained inflow	15.9 ± 3.5	16.4 ± 2.6	0.289
Direct Flow	34.5 ± 3.6	32.9 ± 2.7	0.184
ED KE by component uJ			
Total KE at end diastole uJ	0.57 ± 0.19	0.61 ± 0.27	0.622
Residual volume	0.03 ± 0.02	0.07 ± 0.05	0.030
Delayed ejection flow	0.14 ± 0.06	0.15 ± 0.09	0.420
Retained inflow	0.07 ± 0.04	0.10 ± 0.12	0.123
Direct flow	0.32 ± 0.12	0.35 ± 0.20	0.308

Table 6-2. Whole heart 4D flow components and end-diastolic kinetic energy in AS.

	AS (8)	Control (12)	P value
Gender % male	62.5	66.7	0.180
Age at scan/yrs	61.8 (6.8)	65.6 (7.3)	0.264
Systolic BP mmHg	133.8 (15.8)	133.3 (13.7)	0.950
Diastolic BP mmHg	78.8 (10.3)	73.7 (8.0)	0.259
LVEDVi mls/m^2	76.1 (17.9)	80.2 (6.4)	0.548
SVi mls/m^2	48.8 (12.0)	53.9 (4.5)	0.280
LVEF %	69.9(11.9)	67.3 (4.7)	0.574
Flow components %			
Residual volume	30.2 (5.8)	30.0 (3.7)	0.928
Delayed ejection flow	20.1 (6.1)	16.4 (3.7)	0.157
Retained inflow	16.2 (2.8)	14.9 (3.3)	0.333
Direct Flow	33.8 (3.0)	38.8 (3.4)	0.003
ED KE by component uJ			
Total KE at end diastole uJ			
Residual volume	0.053 (0.044)	0.054 (0.038)	0.939
Delayed ejection flow	0.145 (0.074)	0.183 (0.100)	0.335
Retained inflow	0.086 (0.081)	0.100 (0.071)	0.770
Direct flow	0.334 (0.152)	0.516 (0.257)	0.064

Table 6-3. Demographics and 4D flow results of AS and control group.

6.5 DISCUSSION

The work described in this chapter aimed to assess the technical feasibility of acquiring and studying whole heart 4D flow in AS for the first time. Although my pilot data are underpowered, I was unable to demonstrate changes in component flow in AS, between BAV and TAV AS. AS as a whole were compared with healthy volunteers (acquired using the same protocol) which showed a slight deviation in the direct flow component, with marginally higher direct flow in the control group. The results of the control group are in keeping previously published normal values.[96] This may suggest some early changes, with non-significantly higher retained flow and less direct flow occurring in AS and expansion of the numbers tested would be warranted.

The breakdown of end-diastolic KE did not vary between AS groups but the wide standard deviations limit the application of these findings. Further work is warranted in a larger number of participants to ascertain whether similar changes in end-diastolic values equate to changes in exercise capacity, as recently demonstrated by our unit in participants with cardiomyopathy where changes in end-diastolic kinetic profiles are related to LV impairment (measured by EF) and reduction in direct flow predicts worse performance in 6-minute walk tests (unpublished data). Although the energies involved are in themselves tiny, they may signify clinical changes in cardiomyopathies.

Peak VO_2 was slightly higher in participants with TAV AS but the number of subjects studied was insufficiently powered to allow further comment. Perhaps what is most clear is the enormous variation in exercise capacity. While valve area, aortic afterload and

inefficient preservation of kinetic energy are important, there are clearly numerous additional factors, perhaps related to "physiological conditioning and training", that are also of relevance. AS is an increasingly diverse condition as older patients with TAV AS are recognised and treated alongside the younger cohort with BAV AS. The understanding that the myocardial response of these sub-groups may differ and that this is likely to relate (at least in part) to aortic and systemic factors suggests that it would be logical to separate these patients into different groups in research studies.

Acquisition of whole heart 4D flow is feasible in AS but the early protocols are technically challenging, time consuming and difficult to analyse. Experience suggests that the sequencing works better in large hearts (as in dilated cardiomyoapthy) and are more prone to error in subjects with smaller LV size. The sequence acquires very large amounts of data and reconstruction is sensitive to ectopy. Patients need to remain still to avoid misalignment between cine acquisitions and this can be challenging for some, especially those with musculoskeletal discomfort. While whole heart flow shows promise in dilated cardiomyopathy and congenital heart disease, it does not seem to provide useful additional information in AS in its current format.

Measuring the end physiological effect of AS is of more clinical value and the use of CPET seems likely to expand as evidence of its safety and ability to predict clinical and interventional outcomes accumulates.[198-200] Newer technologies bring the chance to further assess the integrated responses of the cardiovascular, pulmonary and musculoskeletal systems. For example, peripheral muscle anaerobic threshold can be measured with the addition of near infrared muscle spectroscopy. CPET is likely to be

used more widely for the detailed assessment of patients with AS (including, for example, their anaesthetic risk), as part of a move towards personalised medical care. However, while better understanding of the triggers for LV decompensation and symptom onset in individual patients is important for efficient targeting of resources, an array of clinical, imaging and biochemical biomarkers will ultimately be needed to simplify this complex field. As we start to understand more about the complex interactions of AS as a systemic disease, straightforward biomarkers seem further away than ever. For this reason, robust assessment of the ultimate physiological effects (as provided by CPET) may ultimately prove to be a more useful clinical tool.

6.6 CONCLUSION

CPET is a useful tool in assessing symptom and cause of physiological limitation in AS. It provides useful prognostic and risk stratification prior to operative intervention. However, whole heart 4D flow is a new development, which requires further sequence development and assessment before it can be considered a useful research adjunct.

7 GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

Describing AVC as a simple spectrum does not adequately cover the complex phenotypes of the disease. At one end, ASC is more common than previously appreciated in populations without known valve disease and carries a particular cardiovascular risk - whether it should be routinely reported on echocardiograms remains unresolved. There is probably no clear health economic argument or clinical indications for follow up as only a minority progress to significant AS. More pertinent is the associated vascular risk and it seems pragmatic to advise that ASC is reported at echocardiography alongside standard cardiovascular risk scoring and appropriate primary prevention, until more longitudinal outcome data is available. Hypertension is a known association with ASC (which I confirm) - once recognised and treated, absolute values of blood pressure do not seem to differ, implying that there is no important difference in response to therapy in those with ASC.

Overall assessment of post-valvular afterload does not differ between participants with ASC and TAV AS, suggesting that ASC is not simply an earlier stage of AS but a distinct entity with an adverse vascular phenotype which develops fully before a smaller proportion go on to develop progressive valve disease i.e. AS. I describe for the first time that flow is more disordered and turbulent kinetic energy higher in participants with ASC compared with controls. These changes might be expected to increase afterload with long-term effects on the LV, although we found no evidence of increased strain in ASC - larger study numbers may be required to improve sensitivity. However, the OxVALVE population data suggest early remodelling in ASC, with a larger and thicker LV, although these are small but suggestive clinical effects.

Future directions in ASC should include consensus definition. Studies to assess if current standard cardiovascular risk scoring (such as Q risk) recognises this population and are sufficiently sensitive to account for the additional risk from ASC are important. Following this, recommendations for reporting, offering primary prevention based on health economic prediction data would be a significant step forward for this important cohort and their attendant cardiovascular risk profile.

BAV and TAV AS are fundamentally different diseases with differing embryology and genetics. My findings challenge the convention to merge these groups in research and clinical practice, given the difference in higher TKE and stroke work for BAV AS. BAV is associated with an aortopathy but the degree to which this is due to an inherent aortic wall abnormality and how much is due to flow and associated WSS abnormalities is a hotly debated field. My findings cannot answer this but suggest that flow difference and overall WSS are not as difference between these groups as was perhaps assumed. I have shown that the degree of WSS and abnormality of flow angle at the level of the mid ascending aorta is similar in both AS groups, suggesting that the tendency to aortic dilatation may be due to aortic wall pathology (although chronicity of altered flow cannot be fully excluded as a contributor) in BAV. Mid ascending aortic distensibility is similar in both TAV and BAV AS (although turbulent kinetic energy is higher in BAV, implying that irreversible pressure loss is higher in this group). LV dimensions and function do not differ in BAV and TAV AS but all measured parameters of systolic strain were (non significantly) worse in BAV AS - further study in larger groups (incorporating longitudinal follow up) would be appropriate.

TKE shows promise in this early work as a marker of post-valvular afterload and a new additional which allows visualisation of areas of turbulence lead me to believe that this is the most promising use of novel CMR sequences. A challenging minority of patients with AS have discrepant echocardiographic findings (with varying symptom status) and TKE sequences may help to understand this, particularly in BAV AS where there are strong correlations with LV mass, volume, longitudinal strain and stroke work. Proposed studies should be sufficiently powered to include multivariate analysis to better define the LV remodelling factors attributable solely to TKE. My current study numbers cannot, for example, exclude that gender, past hypertension or occult coronary disease could be confounders. Notwithstanding, early work suggest TKE holds promise and the sequence could form a useful research tool, particularly within studies evaluating the role of ACE inhibitors and ARBs in the LV response to AS, which could affect both aortic and LV modelling.

Whole heart 4D flow sequencing has shown promise in the research evaluation of dilated cardiomyopathy. However, my experience demonstrates that the acquisition and processing of the data is time consuming with a success rate of only 67% (albeit using an early version of the sequence). Acquisition times and ease of analysis have improved since acquisition of the data for this thesis. However, I do not envisage that, whole heart 4D flow is likely to provide significant additional insights in AS at the present time.

There is a need for more accurate assessment of total afterload in AS and a recognition that BAV AS may impact on LV function at an earlier stage due to afterload and chronicity of disease. This merits further study. However, given the complexity of measures

available it may be that the development of symptoms ultimately remains the most robust clinical parameter. Having used CPET for this thesis and observed its advantages over simple exercise treadmill testing, I hope for an expansion of CPET as an integral component of future clinical and research practice.

APPENDIX: 1ST AUTHOR ABSTRACTS ARISING FROM THIS WORK AND RELATED PUBLICATIONS

7.1.1 Finalist of Young Investigator of the Year Award: 11th British Society of Cardiac Magnetic Resonance Annual Meeting 2016

Peak turbulent kinetic energy assessed by cardiac magnetic resonance correlates better than aortic valve area with left ventricular parameters in aortic stenosis. **Loudon M**, Bissell M, Dyverfeldt P, Carlhall C, Ebbers T, Hess A, Prendergast B, Neubauer S, Myerson S.

7.1.2 Finalist of Young Investigator of the Year Award: British Heart Valve Society 2015

Peak Turbulent Kinetic Energy in Aortic Valve Calcification is not just dependent on Valve Area. **Loudon M**, Bissell M, Dyverfeldt P, Carlhall C, Ebbers T, Hess A, Prendergast B, Neubauer S, Myerson S.

7.1.3 Oral Presentation: SCMR and EuroCMR Conference Nice 2015

Turbulent kinetic energy in the ascending aorta is greater in bicuspid than tricuspid aortic valve stenosis. **Loudon M**, Bissell M, Dyverfeldt P, Carlhall C, Ebbers T, Hess A, Prendergast B, Neubauer S, Myerson S. *Journal of Cardiovascular Magnetic Resonance* 2015, **17**(Suppl 1):O88

7.1.4 Oral Presentation: Heart Valve Society of America, New York 2014

Newly Diagnosed Aortic Sclerosis is associated with Uncontrolled Hypertension: A Nested Case Control Study From The OxVALVE Population Cohort Study. **Loudon M,** Coffey S, d'Arcy J, Kennedy A, Prendergast B.

7.1.5 High Scoring Abstract and Moderated Poster, EuroEcho 2012

Aortic sclerosis is more frequent in patients with a history of a stroke or transient ischaemic attack: new associations identified via a 2000 patient prospective primary care study (OxVALVE). **Loudon M**, d'Arcy J, Arnold L, Reynolds R, Mabbet C, Prendergast BD. *Eur J Echocardiography Abstracts Supplement (2012) 13 (S1), i17*

7.1.6 Moderated Poster, EuroCMR Vienna 2014

Differences in left ventricular geometry and function between patients with bicuspid and tricuspid aortic valve stenosis. **Loudon M**, Bull S, Joseph J, Neubauer S, Prendergast B, Myerson S.

7.1.7 Selected Related Publications

The OxVALVE Population Cohort Study (OxVALVE-PCS). Population screening for undiagnosed valvular heart disease in the elderly - study design and objectives. Coffey S, **Loudon M**, d'Arcy, Mant D, Farmer A, Prendergast B. *Open Heart* 2014;1:1 e000043

Aortic dilatation in bicuspid aortic valve disease: flow pattern is a major contributor and differs with valve fusion type. Bissell M, Hess A, Biasiolli L, Glaze S, **Loudon M**, Pitcher A,

Davies A, Markl M, Barker A, Neubauer S, Myerson S. *Circ Cardiovasc Imaging* 2013;6 (4):499-507

8 REFERENCES

1. Dweck MR, Boon NA, Newby DE. Calcific aortic stenosis: A disease of the valve and the myocardium. *Journal of the American College of Cardiology*. 2012;60:1854-1863
2. O'Brien KD, Kuusisto J, Reichenbach DD, Ferguson M, Giachelli C, Alpers CE, Otto CM. Osteopontin is expressed in human aortic valvular lesions. *Circulation*. 1995;92:2163-2168
3. Otto CM, Lind BK, Kitzman DW, Gersh BJ, Siscovick DS. Association of aortic-valve sclerosis with cardiovascular mortality and morbidity in the elderly. *The New England journal of medicine*. 1999;341:142-147
4. Coffey S, Cox B, Williams MJ. The prevalence, incidence, progression, and risks of aortic valve sclerosis: A systematic review and meta-analysis. *Journal of the American College of Cardiology*. 2014;63:2852-2861
5. Cosmi JE, Kort S, Tunick PA, Rosenzweig BP, Freedberg RS, Katz ES, Applebaum RM, Kronzon I. The risk of the development of aortic stenosis in patients with "benign" aortic valve thickening. *Archives of internal medicine*. 2002;162:2345-2347
6. Frank S, Johnson A, Ross J, Jr. Natural history of valvular aortic stenosis. *British heart journal*. 1973;35:41-46
7. Basso C, Boschello M, Perrone C, Mecenero A, Cera A, Bicego D, Thiene G, De Dominicis E. An echocardiographic survey of primary school children for bicuspid aortic valve. *The American journal of cardiology*. 2004;93:661-663
8. Fedak PW, Verma S, David TE, Leask RL, Weisel RD, Butany J. Clinical and pathophysiological implications of a bicuspid aortic valve. *Circulation*. 2002;106:900-904
9. Ward C. Clinical significance of the bicuspid aortic valve. *Heart*. 2000;83:81-85
10. Roberts WC, Ko JM. Frequency by decades of unicuspid, bicuspid, and tricuspid aortic valves in adults having isolated aortic valve replacement for aortic stenosis, with or without associated aortic regurgitation. *Circulation*. 2005;111:920-925
11. Beppu S, Suzuki S, Matsuda H, Ohmori F, Nagata S, Miyatake K. Rapidity of progression of aortic stenosis in patients with congenital bicuspid aortic valves. *The American journal of cardiology*. 1993;71:322-327
12. Beroukhim RS, Kruzick TL, Taylor AL, Gao D, Yetman AT. Progression of aortic dilation in children with a functionally normal bicuspid aortic valve. *The American journal of cardiology*. 2006;98:828-830
13. Gurvitz M, Chang RK, Drant S, Allada V. Frequency of aortic root dilation in children with a bicuspid aortic valve. *The American journal of cardiology*. 2004;94:1337-1340
14. La Canna G, Ficarra E, Tsagalau E, Nardi M, Morandini A, Chieffo A, Maisano F, Alfieri O. Progression rate of ascending aortic dilation in patients with normally functioning bicuspid and tricuspid aortic valves. *The American journal of cardiology*. 2006;98:249-253
15. Bissell MM, Hess AT, Biasiolli L, Glaze SJ, Loudon M, Pitcher A, Davis A, Prendergast B, Markl M, Barker AJ, Neubauer S, Myerson SG. Aortic dilation in bicuspid aortic valve disease: Flow pattern is a major contributor and differs with valve fusion type. *Circulation. Cardiovascular imaging*. 2013;6:499-507
16. Hope MD, Hope TA, Meadows AK, Ordovas KG, Urbania TH, Alley MT, Higgins CB. Bicuspid aortic valve: Four-dimensional mr evaluation of ascending aortic systolic flow patterns. *Radiology*. 2010;255:53-61
17. Kurt M, Tanboga IH, Bilen E, Isik T, Kaya A, Karakas MF, Buyukkaya E. Abnormal left ventricular mechanics in isolated bicuspid aortic valve disease may be independent of

aortic distensibility: 2d strain imaging study. *The Journal of heart valve disease*. 2012;21:608-614
18. Kocabay G, Karabay CY, Kalkan S, Kalayci A, Efe SC, Akgun T, Bakal RB, Demir S, Izgi A, Kirma C. Relationship between left ventricular diastolic function and arterial stiffness in patients with bicuspid aortic valve. *The Journal of heart valve disease*. 2014;23:279-288
19. Rajamannan NM, Evans FJ, Aikawa E, Grande-Allen KJ, Demer LL, Heistad DD, Simmons CA, Masters KS, Mathieu P, O'Brien KD, Schoen FJ, Towler DA, Yoganathan AP, Otto CM. Calcific aortic valve disease: Not simply a degenerative process: A review and agenda for research from the national heart and lung and blood institute aortic stenosis working group. Executive summary: Calcific aortic valve disease-2011 update. *Circulation*. 2011;124:1783-1791
20. Barker AJ, Markl M, Burk J, Lorenz R, Bock J, Bauer S, Schulz-Menger J, von Knobelsdorff-Brenkenhoff F. Bicuspid aortic valve is associated with altered wall shear stress in the ascending aorta. *Circulation. Cardiovascular imaging*. 2012;5:457-466
21. Jackson V, Olsson C, Eriksson P, Franco-Cereceda A. Aortic dimensions in patients with bicuspid and tricuspid aortic valves. *The Journal of thoracic and cardiovascular surgery*. 2013;146:605-610
22. Roberts WC. The congenitally bicuspid aortic valve. A study of 85 autopsy cases. *The American journal of cardiology*. 1970;26:72-83
23. Braverman AC. Bicuspid aortic valve and associated aortic wall abnormalities. *Current opinion in cardiology*. 1996;11:501-503
24. de Sa M, Moshkovitz Y, Butany J, David TE. Histologic abnormalities of the ascending aorta and pulmonary trunk in patients with bicuspid aortic valve disease: Clinical relevance to the ross procedure. *The Journal of thoracic and cardiovascular surgery*. 1999;118:588-594
25. Mahadevia R, Barker AJ, Schnell S, Entezari P, Kansal P, Fedak PW, Malaisrie SC, McCarthy P, Collins J, Carr J, Markl M. Bicuspid aortic cusp fusion morphology alters aortic three-dimensional outflow patterns, wall shear stress, and expression of aortopathy. *Circulation*. 2014;129:673-682
26. Guzzardi DG, Barker AJ, van Ooij P, Malaisrie SC, Puthumana JJ, Belke DD, Mewhort HE, Svystonyuk DA, Kang S, Verma S, Collins J, Carr J, Bonow RO, Markl M, Thomas JD, McCarthy PM, Fedak PW. Valve-related hemodynamics mediate human bicuspid aortopathy: Insights from wall shear stress mapping. *Journal of the American College of Cardiology*. 2015;66:892-900
27. Dweck MR, Joshi NV, Rudd JH, Newby DE. Imaging of inflammation and calcification in aortic stenosis. *Current cardiology reports*. 2013;15:320
28. Mohler ER, 3rd, Gannon F, Reynolds C, Zimmerman R, Keane MG, Kaplan FS. Bone formation and inflammation in cardiac valves. *Circulation*. 2001;103:1522-1528
29. Otto CM, Kuusisto J, Reichenbach DD, Gown AM, O'Brien KD. Characterization of the early lesion of 'degenerative' valvular aortic stenosis. Histological and immunohistochemical studies. *Circulation*. 1994;90:844-853
30. O'Brien KD, Reichenbach DD, Marcovina SM, Kuusisto J, Alpers CE, Otto CM. Apolipoproteins b, (a), and e accumulate in the morphologically early lesion of 'degenerative' valvular aortic stenosis. *Arteriosclerosis, thrombosis, and vascular biology*. 1996;16:523-532
31. Michelena HI, Desjardins VA, Avierinos JF, Russo A, Nkomo VT, Sundt TM, Pellikka PA, Tajik AJ, Enriquez-Sarano M. Natural history of asymptomatic patients with normally functioning or minimally dysfunctional bicuspid aortic valve in the community. *Circulation*. 2008;117:2776-2784
32. Chandra S, Rajamannan NM, Sucosky P. Computational assessment of bicuspid aortic valve wall-shear stress: Implications for calcific aortic valve disease. *Biomechanics and modeling in mechanobiology*. 2012;11:1085-1096

33. Jermihov PN, Jia L, Sacks MS, Gorman RC, Gorman JH, 3rd, Chandran KB. Effect of geometry on the leaflet stresses in simulated models of congenital bicuspid aortic valves. *Cardiovascular engineering and technology*. 2011;2:48-56
34. Tuseth N, Cramariuc D, Rieck AE, Wachtell K, Gerdts E. Asymmetric septal hypertrophy - a marker of hypertension in aortic stenosis (a seas substudy). *Blood pressure*. 2010;19:140-144
35. Yotti R, Bermejo J. Left ventricular hypertrophy in aortic valve stenosis: Friend or foe? *Heart*. 2011;97:269-271
36. Dweck MR, Joshi S, Murigu T, Gulati A, Alpendurada F, Jabbour A, Maceira A, Roussin I, Northridge DB, Kilner PJ, Cook SA, Boon NA, Pepper J, Mohiaddin RH, Newby DE, Pennell DJ, Prasad SK. Left ventricular remodeling and hypertrophy in patients with aortic stenosis: Insights from cardiovascular magnetic resonance. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2012;14:50
37. Milavetz DL, Hayes SN, Weston SA, Seward JB, Mullany CJ, Roger VL. Sex differences in left ventricular geometry in aortic stenosis: Impact on outcome. *Chest*. 2000;117:1094-1099
38. Selvetella G, Hirsch E, Notte A, Tarone G, Lembo G. Adaptive and maladaptive hypertrophic pathways: Points of convergence and divergence. *Cardiovascular research*. 2004;63:373-380
39. Esposito G, Rapacciuolo A, Naga Prasad SV, Takaoka H, Thomas SA, Koch WJ, Rockman HA. Genetic alterations that inhibit in vivo pressure-overload hypertrophy prevent cardiac dysfunction despite increased wall stress. *Circulation*. 2002;105:85-92
40. Olson EN, Williams RS. Calcineurin signaling and muscle remodeling. *Cell*. 2000;101:689-692
41. Crabtree GR. Generic signals and specific outcomes: Signaling through ca2+, calcineurin, and nf-at. *Cell*. 1999;96:611-614
42. Hill JA, Rothermel B, Yoo KD, Cabuay B, Demetroulis E, Weiss RM, Kutschke W, Bassel-Duby R, Williams RS. Targeted inhibition of calcineurin in pressure-overload cardiac hypertrophy. Preservation of systolic function. *The Journal of biological chemistry*. 2002;277:10251-10255
43. Paradis P, Dali-Youcef N, Paradis FW, Thibault G, Nemer M. Overexpression of angiotensin ii type i receptor in cardiomyocytes induces cardiac hypertrophy and remodeling. *Proceedings of the National Academy of Sciences of the United States of America*. 2000;97:931-936
44. Esposito G, Rapacciuolo A, Naga Prasad SV, Rockman HA. Cardiac hypertrophy: Role of g protein-coupled receptors. *Journal of cardiac failure*. 2002;8:S409-414
45. Nichols WW, O'Rourke MF, Avolio AP, Yaginuma T, Murgo JP, Pepine CJ, Conti CR. Effects of age on ventricular-vascular coupling. *The American journal of cardiology*. 1985;55:1179-1184
46. O'Rourke MF, Nichols WW. Aortic diameter, aortic stiffness, and wave reflection increase with age and isolated systolic hypertension. *Hypertension*. 2005;45:652-658
47. Roman MJ, Okin PM, Kizer JR, Lee ET, Howard BV, Devereux RB. Relations of central and brachial blood pressure to left ventricular hypertrophy and geometry: The strong heart study. *Journal of hypertension*. 2010;28:384-388
48. London GM, Pannier B, Guerin AP, Marchais SJ, Safar ME, Cuche JL. Cardiac hypertrophy, aortic compliance, peripheral resistance, and wave reflection in end-stage renal disease. Comparative effects of ace inhibition and calcium channel blockade. *Circulation*. 1994;90:2786-2796
49. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R, Prospective Studies C. Age-specific relevance of usual blood pressure to vascular mortality: A meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*. 2002;360:1903-1913

50. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, Amann M, Anderson HR, Andrews KG, Aryee M, Atkinson C, Bacchus LJ, Bahalim AN, Balakrishnan K, Balmes J, Barker-Collo S, Baxter A, Bell ML, Blore JD, Blyth F, Bonner C, Borges G, Bourne R, Boussinesq M, Brauer M, Brooks P, Bruce NG, Brunekreef B, Bryan-Hancock C, Bucello C, Buchbinder R, Bull F, Burnett RT, Byers TE, Calabria B, Carapetis J, Carnahan E, Chafe Z, Charlson F, Chen H, Chen JS, Cheng AT, Child JC, Cohen A, Colson KE, Cowie BC, Darby S, Darling S, Davis A, Degenhardt L, Dentener F, Des Jarlais DC, Devries K, Dherani M, Ding EL, Dorsey ER, Driscoll T, Edmond K, Ali SE, Engell RE, Erwin PJ, Fahimi S, Falder G, Farzadfar F, Ferrari A, Finucane MM, Flaxman S, Fowkes FG, Freedman G, Freeman MK, Gakidou E, Ghosh S, Giovannucci E, Gmel G, Graham K, Grainger R, Grant B, Gunnell D, Gutierrez HR, Hall W, Hoek HW, Hogan A, Hosgood HD, 3rd, Hoy D, Hu H, Hubbell BJ, Hutchings SJ, Ibeanusi SE, Jacklyn GL, Jasrasaria R, Jonas JB, Kan H, Kanis JA, Kassebaum N, Kawakami N, Khang YH, Khatibzadeh S, Khoo JP, Kok C, Laden F, Lalloo R, Lan Q, Lathlean T, Leasher JL, Leigh J, Li Y, Lin JK, Lipshultz SE, London S, Lozano R, Lu Y, Mak J, Malekzadeh R, Mallinger L, Marcenes W, March L, Marks R, Martin R, McGale P, McGrath J, Mehta S, Mensah GA, Merriman TR, Micha R, Michaud C, Mishra V, Mohd Hanafiah K, Mokdad AA, Morawska L, Mozaffarian D, Murphy T, Naghavi M, Neal B, Nelson PK, Nolla JM, Norman R, Olives C, Omer SB, Orchard J, Osborne R, Ostro B, Page A, Pandey KD, Parry CD, Passmore E, Patra J, Pearce N, Pelizzari PM, Petzold M, Phillips MR, Pope D, Pope CA, 3rd, Powles J, Rao M, Razavi H, Rehfuess EA, Rehm JT, Ritz B, Rivara FP, Roberts T, Robinson C, Rodriguez-Portales JA, Romieu I, Room R, Rosenfeld LC, Roy A, Rushton L, Salomon JA, Sampson U, Sanchez-Riera L, Sanman E, Sapkota A, Seedat S, Shi P, Shield K, Shivakoti R, Singh GM, Sleet DA, Smith E, Smith KR, Stapelberg NJ, Steenland K, Stockl H, Stovner LJ, Straif K, Straney L, Thurston GD, Tran JH, Van Dingenen R, van Donkelaar A, Veerman JL, Vijayakumar L, Weintraub R, Weissman MM, White RA, Whiteford H, Wiersma ST, Wilkinson JD, Williams HC, Williams W, Wilson N, Woolf AD, Yip P, Zielinski JM, Lopez AD, Murray CJ, Ezzati M, AlMazroa MA, Memish ZA. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: A systematic analysis for the global burden of disease study 2010. *Lancet*. 2012;380:2224-2260
51. Roman MJ, Devereux RB, Kizer JR, Lee ET, Galloway JM, Ali T, Umans JG, Howard BV. Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: The strong heart study. *Hypertension*. 2007;50:197-203
52. Jankowski P, Kawecka-Jaszcz K, Czarnecka D, Brzozowska-Kiszka M, Styczkiewicz K, Loster M, Kloch-Badelek M, Wilinski J, Curylo AM, Dudek D, Aortic Blood P, Survival Study G. Pulsatile but not steady component of blood pressure predicts cardiovascular events in coronary patients. *Hypertension*. 2008;51:848-855
53. Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, Hughes AD, Thurston H, O'Rourke M, Investigators C, Anglo-Scandinavian Cardiac Outcomes Trial I, Committee CS, Writing C. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: Principal results of the conduit artery function evaluation (cafe) study. *Circulation*. 2006;113:1213-1225
54. McEniery CM, Cockcroft JR, Roman MJ, Franklin SS, Wilkinson IB. Central blood pressure: Current evidence and clinical importance. *European heart journal*. 2014;35:1719-1725
55. Pucci G, Cheriyan J, Hubsch A, Hickson SS, Gajendragadkar PR, Watson T, O'Sullivan M, Woodcock-Smith J, Schillaci G, Wilkinson IB, McEniery CM. Evaluation of the vicorder, a novel cuff-based device for the noninvasive estimation of central blood pressure. *Journal of hypertension*. 2013;31:77-85
56. Briand M, Dumesnil JG, Kadem L, Tongue AG, Rieu R, Garcia D, Pibarot P. Reduced systemic arterial compliance impacts significantly on left ventricular afterload and function

in aortic stenosis: Implications for diagnosis and treatment. *Journal of the American College of Cardiology*. 2005;46:291-298
57. Rieck AE, Cramariuc D, Boman K, Gohlke-Barwolf C, Staal EM, Lonnebakken MT, Rossebo AB, Gerdts E. Hypertension in aortic stenosis: Implications for left ventricular structure and cardiovascular events. *Hypertension*. 2012;60:90-97
58. Lancellotti P, Donal E, Magne J, Moonen M, O'Connor K, Daubert JC, Pierard LA. Risk stratification in asymptomatic moderate to severe aortic stenosis: The importance of the valvular, arterial and ventricular interplay. *Heart*. 2010;96:1364-1371
59. Cramariuc D, Cioffi G, Rieck AE, Devereux RB, Staal EM, Ray S, Wachtell K, Gerdts E. Low-flow aortic stenosis in asymptomatic patients: Valvular-arterial impedance and systolic function from the seas substudy. *JACC. Cardiovascular imaging*. 2009;2:390-399
60. Lancellotti P, Donal E, Magne J, O'Connor K, Moonen ML, Cosyns B, Pierard LA. Impact of global left ventricular afterload on left ventricular function in asymptomatic severe aortic stenosis: A two-dimensional speckle-tracking study. *European journal of echocardiography : the journal of the Working Group on Echocardiography of the European Society of Cardiology*. 2010;11:537-543
61. Maroules CD, Khera A, Ayers C, Goel A, Peshock RM, Abbara S, King KS. Cardiovascular outcome associations among cardiovascular magnetic resonance measures of arterial stiffness: The dallas heart study. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2014;16:33
62. Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, Asmar R, Reneman RS, Hoeks AP, Breteler MM, Witteman JC. Arterial stiffness and risk of coronary heart disease and stroke: The rotterdam study. *Circulation*. 2006;113:657-663
63. Kidher E, Harling L, Ashrafian H, Naase H, Francis DP, Evans P, Athanasiou T. Aortic stiffness as a marker of cardiac function and myocardial strain in patients undergoing aortic valve replacement. *Journal of cardiothoracic surgery*. 2014;9:102
64. Kidher E, Harling L, Nihoyannopoulos P, Shenker N, Ashrafian H, Francis DP, Mayet J, Athanasiou T. High aortic pulse wave velocity is associated with poor quality of life in surgical aortic valve stenosis patients. *Interactive cardiovascular and thoracic surgery*. 2014;19:189-197
65. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension*. 2001;37:1236-1241
66. Sutton-Tyrrell K, Najjar SS, Boudreau RM, Venkitachalam L, Kupelian V, Simonsick EM, Havlik R, Lakatta EG, Spurgeon H, Kritchevsky S, Pahor M, Bauer D, Newman A, Health ABCS. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. *Circulation*. 2005;111:3384-3390
67. Moaref A, Khavanin M, Shekarforoush S. Aortic distensibility in bicuspid aortic valve patients with normal aortic diameter. *Therapeutic advances in cardiovascular disease*. 2014;8:128-132
68. Nistri S, Grande-Allen J, Noale M, Basso C, Siviero P, Maggi S, Crepaldi G, Thiene G. Aortic elasticity and size in bicuspid aortic valve syndrome. *European heart journal*. 2008;29:472-479
69. Garcia D, Pibarot P, Dumesnil JG, Sakr F, Durand LG. Assessment of aortic valve stenosis severity: A new index based on the energy loss concept. *Circulation*. 2000;101:765-771
70. Pibarot P, Garcia D, Dumesnil JG. Energy loss index in aortic stenosis: From fluid mechanics concept to clinical application. *Circulation*. 2013;127:1101-1104
71. Bahlmann E, Gerdts E, Cramariuc D, Gohlke-Baerwolf C, Nienaber CA, Wachtell K, Seifert R, Chambers JB, Kuck KH, Ray S. Prognostic value of energy loss index in asymptomatic aortic stenosis. *Circulation*. 2013;127:1149-1156

72. Dyverfeldt P, Hope MD, Tseng EE, Saloner D. Magnetic resonance measurement of turbulent kinetic energy for the estimation of irreversible pressure loss in aortic stenosis. *JACC. Cardiovascular imaging*. 2013;6:64-71
73. Sasayama S, Ross J, Jr., Franklin D, Bloor CM, Bishop S, Dilley RB. Adaptations of the left ventricle to chronic pressure overload. *Circulation research*. 1976;38:172-178
74. Cioffi G, Faggiano P, Vizzardi E, Tarantini L, Cramariuc D, Gerdts E, de Simone G. Prognostic effect of inappropriately high left ventricular mass in asymptomatic severe aortic stenosis. *Heart*. 2011;97:301-307
75. Otto CM, Burwash IG, Legget ME, Munt BI, Fujioka M, Healy NL, Kraft CD, Miyake-Hull CY, Schwaegler RG. Prospective study of asymptomatic valvular aortic stenosis. Clinical, echocardiographic, and exercise predictors of outcome. *Circulation*. 1997;95:2262-2270
76. Chin CW, Shah AS, Vassiliou V, Cowell SJ, Doris M, Kwok TC, Semple S, Zamvar V, White AC, McKillop G, Boon NA, Prasad SK, Mills NL, Newby DE, Dweck MR. Left ventricular hypertrophy with strain and aortic stenosis. *Circulation*. 2014
77. Carroll JD, Carroll EP, Feldman T, Ward DM, Lang RM, McGaughey D, Karp RB. Sex-associated differences in left ventricular function in aortic stenosis of the elderly. *Circulation*. 1992;86:1099-1107
78. Capoulade R, Clavel MA, Dumesnil JG, Chan KL, Teo KK, Tam JW, Cote N, Mathieu P, Despres JP, Pibarot P, Investigators A. Insulin resistance and lvh progression in patients with calcific aortic stenosis: A substudy of the astronomer trial. *JACC. Cardiovascular imaging*. 2013;6:165-174
79. Weidemann F, Herrmann S, Stork S, Niemann M, Frantz S, Lange V, Beer M, Gattenlohner S, Voelker W, Ertl G, Strotmann JM. Impact of myocardial fibrosis in patients with symptomatic severe aortic stenosis. *Circulation*. 2009;120:577-584
80. Dweck MR, Joshi S, Murigu T, Alpendurada F, Jabbour A, Melina G, Banya W, Gulati A, Roussin I, Raza S, Prasad NA, Wage R, Quarto C, Angeloni E, Refice S, Sheppard M, Cook SA, Kilner PJ, Pennell DJ, Newby DE, Mohiaddin RH, Pepper J, Prasad SK. Midwall fibrosis is an independent predictor of mortality in patients with aortic stenosis. *Journal of the American College of Cardiology*. 2011;58:1271-1279
81. Reant P, Lederlin M, Lafitte S, Serri K, Montaudon M, Corneloup O, Roudaut R, Laurent F. Absolute assessment of aortic valve stenosis by planimetry using cardiovascular magnetic resonance imaging: Comparison with transesophageal echocardiography, transthoracic echocardiography, and cardiac catheterisation. *European journal of radiology*. 2006;59:276-283
82. Chuang ML, Hibberd MG, Salton CJ, Beaudin RA, Riley MF, Parker RA, Douglas PS, Manning WJ. Importance of imaging method over imaging modality in noninvasive determination of left ventricular volumes and ejection fraction: Assessment by two- and three-dimensional echocardiography and magnetic resonance imaging. *Journal of the American College of Cardiology*. 2000;35:477-484
83. Pennell DJ. Cardiovascular magnetic resonance: Twenty-first century solutions in cardiology. *Clinical medicine*. 2003;3:273-278
84. Burman ED, Keegan J, Kilner PJ. Aortic root measurement by cardiovascular magnetic resonance: Specification of planes and lines of measurement and corresponding normal values. *Circulation. Cardiovascular imaging*. 2008;1:104-113
85. Voges I, Jerosch-Herold M, Hedderich J, Pardun E, Hart C, Gabbert DD, Hansen JH, Petko C, Kramer HH, Rickers C. Normal values of aortic dimensions, distensibility, and pulse wave velocity in children and young adults: A cross-sectional study. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2012;14:77
86. Nethononda RM, Lewandowski AJ, Stewart R, Kylinterias I, Whitworth P, Francis J, Leeson P, Watkins H, Neubauer S, Rider OJ. Gender specific patterns of age-related decline in aortic stiffness: A cardiovascular magnetic resonance study including normal

ranges. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2015;17:20

87. Vahanian A, Alfieri O, Andreotti F, Antunes MJ, Baron-Esquivias G, Baumgartner H, Borger MA, Carrel TP, De Bonis M, Evangelista A, Falk V, Lung B, Lancellotti P, Pierard L, Price S, Schafers HJ, Schuler G, Stepinska J, Swedberg K, Takkenberg J, Von Oppell UO, Windecker S, Zamorano JL, Zembala M, Guidelines ESCCfP, Joint Task Force on the Management of Valvular Heart Disease of the European Society of C, European Association for Cardio-Thoracic S. Guidelines on the management of valvular heart disease (version 2012): The joint task force on the management of valvular heart disease of the european society of cardiology (esc) and the european association for cardio-thoracic surgery (eacts). *European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery*. 2012;42:S1-44
88. Lee HF, Hsu LA, Chan YH, Wang CL, Chang CJ, Kuo CT. Prognostic value of global left ventricular strain for conservatively treated patients with symptomatic aortic stenosis. *Journal of cardiology*. 2013;62:301-306
89. Delgado V, Tops LF, van Bommel RJ, van der Kley F, Marsan NA, Klautz RJ, Versteegh MI, Holman ER, Schalij MJ, Bax JJ. Strain analysis in patients with severe aortic stenosis and preserved left ventricular ejection fraction undergoing surgical valve replacement. *European heart journal*. 2009;30:3037-3047
90. Carasso S, Mutlak D, Lessick J, Reisner SA, Rakowski H, Agmon Y. Symptoms in severe aortic stenosis are associated with decreased compensatory circumferential myocardial mechanics. *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography*. 2015;28:218-225
91. Carasso S, Cohen O, Mutlak D, Adler Z, Lessick J, Aronson D, Reisner SA, Rakowski H, Bolotin G, Agmon Y. Relation of myocardial mechanics in severe aortic stenosis to left ventricular ejection fraction and response to aortic valve replacement. *The American journal of cardiology*. 2011;107:1052-1057
92. Singh A, Steadman CD, Khan JN, Horsfield MA, Bekele S, Nazir SA, Kanagala P, Masca NG, Clarysse P, McCann GP. Intertechnique agreement and interstudy reproducibility of strain and diastolic strain rate at 1.5 and 3 tesla: A comparison of feature-tracking and tagging in patients with aortic stenosis. *Journal of magnetic resonance imaging : JMRI*. 2015;41:1129-1137
93. Khan JN, Singh A, Nazir SA, Kanagala P, Gershlick AH, McCann GP. Comparison of cardiovascular magnetic resonance feature tracking and tagging for the assessment of left ventricular systolic strain in acute myocardial infarction. *European journal of radiology*. 2015;84:840-848
94. Markl M, Frydrychowicz A, Kozerke S, Hope M, Wieben O. 4d flow mri. *Journal of magnetic resonance imaging : JMRI*. 2012;36:1015-1036
95. Barker AJ, van Ooij P, Bandi K, Garcia J, Albaghdadi M, McCarthy P, Bonow RO, Carr J, Collins J, Malaisrie SC, Markl M. Viscous energy loss in the presence of abnormal aortic flow. *Magnetic resonance in medicine*. 2014;72:620-628
96. Eriksson J, Bolger AF, Ebbers T, Carlhall CJ. Four-dimensional blood flow-specific markers of lv dysfunction in dilated cardiomyopathy. *European heart journal cardiovascular Imaging*. 2013;14:417-424
97. Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP, 3rd, Guyton RA, O'Gara PT, Ruiz CE, Skubas NJ, Sorajja P, Sundt TM, 3rd, Thomas JD, American College of Cardiology/American Heart Association Task Force on Practice G. 2014 aha/acc guideline for the management of patients with valvular heart disease: A report of the american college of cardiology/american heart association task force on practice guidelines. *Journal of the American College of Cardiology*. 2014;63:e57-185
98. Levy F, Fayad N, Jeu A, Choquet D, Szymanski C, Malaquin D, Peltier M, Tribouilloy C. The value of cardiopulmonary exercise testing in individuals with apparently

asymptomatic severe aortic stenosis: A pilot study. *Archives of cardiovascular diseases*. 2014;107:519-528
99. Dhoble A, Enriquez-Sarano M, Kopecky SL, Abdelmoneim SS, Cruz P, Thomas RJ, Allison TG. Cardiopulmonary responses to exercise and its utility in patients with aortic stenosis. *The American journal of cardiology*. 2014;113:1711-1716
100. Coffey S, d'Arcy JL, Loudon MA, Mant D, Farmer AJ, Prendergast BD, Ox V-PCSg. The oxvalve population cohort study (oxvalve-pcs)-population screening for undiagnosed valvular heart disease in the elderly: Study design and objectives. *Open heart*. 2014;1:e000043
101. Baumgartner H, Hung J, Bermejo J, Chambers JB, Evangelista A, Griffin BP, Iung B, Otto CM, Pellikka PA, Quinones M, Eae/Ase. Echocardiographic assessment of valve stenosis: Eae/ase recommendations for clinical practice. *European journal of echocardiography : the journal of the Working Group on Echocardiography of the European Society of Cardiology*. 2009;10:1-25
102. Kracht D, Shroff R, Baig S, Doyon A, Jacobi C, Zeller R, Querfeld U, Schaefer F, Wuhl E, Schmidt BM, Melk A, Consortium CS. Validating a new oscillometric device for aortic pulse wave velocity measurements in children and adolescents. *American journal of hypertension*. 2011;24:1294-1299
103. Shahin Y, Barakat H, Barnes R, Chetter I. The vicorder device compared with sphygmocor in the assessment of carotid-femoral pulse wave velocity in patients with peripheral arterial disease. *Hypertension research : official journal of the Japanese Society of Hypertension*. 2013;36:208-212
104. Hess AT, Bissell MM, Ntusi NA, Lewis AJ, Tunnicliffe EM, Greiser A, Stalder AF, Francis JM, Myerson SG, Neubauer S, Robson MD. Aortic 4d flow: Quantification of signal-to-noise ratio as a function of field strength and contrast enhancement for 1.5t, 3t, and 7t. *Magnetic resonance in medicine*. 2015;73:1864-1871
105. Pennell DJ, Sechtem UP, Higgins CB, Manning WJ, Pohost GM, Rademakers FE, van Rossum AC, Shaw LJ, Yucel EK, European Society of c, Soceity for Cardiovascular Magnetic R. Clinical indications for cardiovascular magnetic resonance (cmr): Consensus panel report. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2004;6:727-765
106. Maret E, Todt T, Brudin L, Nylander E, Swahn E, Ohlsson JL, Engvall JE. Functional measurements based on feature tracking of cine magnetic resonance images identify left ventricular segments with myocardial scar. *Cardiovascular ultrasound*. 2009;7:53
107. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ, Chamber Quantification Writing G, American Society of Echocardiography's G, Standards C, European Association of E. Recommendations for chamber quantification: A report from the american society of echocardiography's guidelines and standards committee and the chamber quantification writing group, developed in conjunction with the european association of echocardiography, a branch of the european society of cardiology. *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography*. 2005;18:1440-1463
108. Jiamsripong P, Honda T, Reuss CS, Hurst RT, Chaliki HP, Grill DE, Schneck SL, Tyler R, Khandheria BK, Lester SJ. Three methods for evaluation of left atrial volume. *European journal of echocardiography : the journal of the Working Group on Echocardiography of the European Society of Cardiology*. 2008;9:351-355
109. Nacif MS, Barranhas AD, Turkbey E, Marchiori E, Kawel N, Mello RA, Falcao RO, Oliveira AC, Jr., Rochitte CE. Left atrial volume quantification using cardiac mri in atrial fibrillation: Comparison of the simpson's method with biplane area-length, ellipse, and three-dimensional methods. *Diagnostic and interventional radiology*. 2013;19:213-220

110. O'Brien KR, Cowan BR, Jain M, Stewart RA, Kerr AJ, Young AA. Mri phase contrast velocity and flow errors in turbulent stenotic jets. *Journal of magnetic resonance imaging : JMRI*. 2008;28:210-218
111. Hiratzka LF, Bakris GL, Beckman JA, Bersin RM, Carr VF, Casey DE, Jr., Eagle KA, Hermann LK, Isselbacher EM, Kazerooni EA, Kouchoukos NT, Lytle BW, Milewicz DM, Reich DL, Sen S, Shinn JA, Svensson LG, Williams DM, American College of Cardiology Foundation/American Heart Association Task Force on Practice G, American Association for Thoracic S, American College of R, American Stroke A, Society of Cardiovascular A, Society for Cardiovascular A, Interventions, Society of Interventional R, Society of Thoracic S, Society for Vascular M. 2010 accf/aha/aats/acr/asa/sca/scai/sir/sts/svm guidelines for the diagnosis and management of patients with thoracic aortic disease: A report of the american college of cardiology foundation/american heart association task force on practice guidelines, american association for thoracic surgery, american college of radiology, american stroke association, society of cardiovascular anesthesiologists, society for cardiovascular angiography and interventions, society of interventional radiology, society of thoracic surgeons, and society for vascular medicine. *Circulation*. 2010;121:e266-369
112. Haycock GB, Schwartz GJ, Wisotsky DH. Geometric method for measuring body surface area: A height-weight formula validated in infants, children, and adults. *The Journal of pediatrics*. 1978;93:62-66
113. Jackson CE, Shirodaria CC, Lee JM, Francis JM, Choudhury RP, Channon KM, Noble JA, Neubauer S, Robson MD. Reproducibility and accuracy of automated measurement for dynamic arterial lumen area by cardiovascular magnetic resonance. *The international journal of cardiovascular imaging*. 2009;25:797-808
114. Dogui A, Redheuil A, Lefort M, DeCesare A, Kachenoura N, Herment A, Mousseaux E. Measurement of aortic arch pulse wave velocity in cardiovascular mr: Comparison of transit time estimators and description of a new approach. *Journal of magnetic resonance imaging : JMRI*. 2011;33:1321-1329
115. Dogui A, Kachenoura N, Frouin F, Lefort M, De Cesare A, Mousseaux E, Herment A. Consistency of aortic distensibility and pulse wave velocity estimates with respect to the bramwell-hill theoretical model: A cardiovascular magnetic resonance study. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2011;13:11
116. Stalder AF, Russe MF, Frydrychowicz A, Bock J, Hennig J, Markl M. Quantitative 2d and 3d phase contrast mri: Optimized analysis of blood flow and vessel wall parameters. *Magnetic resonance in medicine*. 2008;60:1218-1231
117. Sigovan M, Hope MD, Dyverfeldt P, Saloner D. Comparison of four-dimensional flow parameters for quantification of flow eccentricity in the ascending aorta. *Journal of magnetic resonance imaging : JMRI*. 2011;34:1226-1230
118. Markl M, Wallis W, Harloff A. Reproducibility of flow and wall shear stress analysis using flow-sensitive four-dimensional mri. *Journal of magnetic resonance imaging : JMRI*. 2011;33:988-994
119. Dyverfeldt P, Gardhagen R, Sigfridsson A, Karlsson M, Ebbers T. On mri turbulence quantification. *Magnetic resonance imaging*. 2009;27:913-922
120. Lantz J, Ebbers T, Engvall J, Karlsson M. Numerical and experimental assessment of turbulent kinetic energy in an aortic coarctation. *Journal of biomechanics*. 2013;46:1851-1858
121. Eriksson J, Carlhall CJ, Dyverfeldt P, Engvall J, Bolger AF, Ebbers T. Semi-automatic quantification of 4d left ventricular blood flow. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2010;12:9

122. Heiberg E, Sjogren J, Ugander M, Carlsson M, Engblom H, Arheden H. Design and validation of segment--freely available software for cardiovascular image analysis. *BMC medical imaging*. 2010;10:1
123. Eriksson J, Dyverfeldt P, Engvall J, Bolger AF, Ebbers T, Carlhall CJ. Quantification of presystolic blood flow organization and energetics in the human left ventricle. *American journal of physiology. Heart and circulatory physiology*. 2011;300:H2135-2141
124. Novaro GM, Katz R, Aviles RJ, Gottdiener JS, Cushman M, Psaty BM, Otto CM, Griffin BP. Clinical factors, but not c-reactive protein, predict progression of calcific aortic-valve disease: The cardiovascular health study. *Journal of the American College of Cardiology*. 2007;50:1992-1998
125. Messika-Zeitoun D, Bielak LF, Peyser PA, Sheedy PF, Turner ST, Nkomo VT, Breen JF, Maalouf J, Scott C, Tajik AJ, Enriquez-Sarano M. Aortic valve calcification: Determinants and progression in the population. *Arteriosclerosis, thrombosis, and vascular biology*. 2007;27:642-648
126. Carabello BA. Aortic sclerosis--a window to the coronary arteries? *The New England journal of medicine*. 1999;341:193-195
127. Owens DS, Budoff MJ, Katz R, Takasu J, Shavelle DM, Carr JJ, Heckbert SR, Otto CM, Probstfield JL, Kronmal RA, O'Brien KD. Aortic valve calcium independently predicts coronary and cardiovascular events in a primary prevention population. *JACC. Cardiovascular imaging*. 2012;5:619-625
128. Stritzke J, Linsel-Nitschke P, Markus MR, Mayer B, Lieb W, Luchner A, Doring A, Koenig W, Keil U, Hense HW, Schunkert H, Investigators MK. Association between degenerative aortic valve disease and long-term exposure to cardiovascular risk factors: Results of the longitudinal population-based kora/monica survey. *European heart journal*. 2009;30:2044-2053
129. Owens DS, Katz R, Takasu J, Kronmal R, Budoff MJ, O'Brien KD. Incidence and progression of aortic valve calcium in the multi-ethnic study of atherosclerosis (mesa). *The American journal of cardiology*. 2010;105:701-708
130. Sverdlov AL, Ngo DT, Chan WP, Chirkov YY, Gersh BJ, McNeil JJ, Horowitz JD. Determinants of aortic sclerosis progression: Implications regarding impairment of nitric oxide signalling and potential therapeutics. *European heart journal*. 2012;33:2419-2425
131. Sverdlov AL, Ngo DT, Horowitz JD. Pathogenesis of aortic sclerosis: Association with low bmi, tissue nitric oxide resistance, but not systemic inflammatory activation. *American journal of cardiovascular disease*. 2012;2:43-49
132. El Accaoui RN, Gould ST, Hajj GP, Chu Y, Davis MK, Kraft DC, Lund DD, Brooks RM, Doshi H, Zimmerman KA, Kutschke W, Anseth KS, Heistad DD, Weiss RM. Aortic valve sclerosis in mice deficient in endothelial nitric oxide synthase. *American journal of physiology. Heart and circulatory physiology*. 2014;306:H1302-1313
133. Agno FS, Chinali M, Bella JN, Liu JE, Arnett DK, Kitzman DW, Oberman A, Hopkins PN, Rao DC, Devereux RB. Aortic valve sclerosis is associated with preclinical cardiovascular disease in hypertensive adults: The hypertension genetic epidemiology network study. *Journal of hypertension*. 2005;23:867-873
134. Olsen MH, Wachtell K, Bella JN, Palmieri V, Gerdts E, Smith G, Nieminen MS, Dahlof B, Ibsen H, Devereux RB. Aortic valve sclerosis and albuminuria predict cardiovascular events independently in hypertension: A losartan intervention for endpoint-reduction in hypertension (life) substudy. *American journal of hypertension*. 2005;18:1430-1436
135. da Rosa EM, Sant'anna JR, Oppermann LP, Castro I. Prognosis of aortic valve sclerosis in cardiovascular mortality of patients seen at the cardiology institute of rio grande do sul. *Arquivos brasileiros de cardiologia*. 2007;88:234-239
136. Messika-Zeitoun D, Aubry MC, Detaint D, Bielak LF, Peyser PA, Sheedy PF, Turner ST, Breen JF, Scott C, Tajik AJ, Enriquez-Sarano M. Evaluation and clinical implications of aortic valve calcification measured by electron-beam computed tomography. *Circulation*. 2004;110:356-362

137. Ngo DT, Wuttke RD, Turner S, Marwick TH, Horowitz JD. Quantitative assessment of aortic sclerosis using ultrasonic backscatter. *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography*. 2004;17:1123-1130
138. Teichholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: Echocardiographic-angiographic correlations in the presence of absence of asynergy. *The American journal of cardiology*. 1976;37:7-11
139. Shahgaldi K, Gudmundsson P, Manouras A, Brodin LA, Winter R. Visually estimated ejection fraction by two dimensional and triplane echocardiography is closely correlated with quantitative ejection fraction by real-time three dimensional echocardiography. *Cardiovascular ultrasound*. 2009;7:41
140. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W, Voigt JU. Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the american society of echocardiography and the european association of cardiovascular imaging. *European heart journal cardiovascular Imaging*. 2015;16:233-270
141. Volzke H, Haring R, Lorbeer R, Wallaschofski H, Reffelmann T, Empen K, Rettig R, John U, Felix SB, Dorr M. Heart valve sclerosis predicts all-cause and cardiovascular mortality. *Atherosclerosis*. 2010;209:606-610
142. Agmon Y, Khandheria BK, Meissner I, Sicks JR, O'Fallon WM, Wiebers DO, Whisnant JP, Seward JB, Tajik AJ. Aortic valve sclerosis and aortic atherosclerosis: Different manifestations of the same disease? Insights from a population-based study. *Journal of the American College of Cardiology*. 2001;38:827-834
143. Fried LP, Borhani NO, Enright P, Furberg CD, Gardin JM, Kronmal RA, Kuller LH, Manolio TA, Mittelmark MB, Newman A, et al. The cardiovascular health study: Design and rationale. *Annals of epidemiology*. 1991;1:263-276
144. Korkmaz L, Agac MT, Bektas H, Varol MO, Erkan H, Acar Z, Kurt D, Celik S. Aortic valve sclerosis is a sign of increased arterial stiffness in clinically asymptomatic subjects. *Cardiology journal*. 2013;20:318-322
145. Korkmaz L, Agac MT, Bektas H, Varol MO, Erkan H, Acar Z, Kurt D, Celik S. Relation between aortic valve sclerosis and ankle-brachial index in participants clinically free of atherosclerotic vascular disease. *Angiology*. 2013;64:391-394
146. Celik S, Durmus I, Korkmaz L, Gedikli O, Kaplan S, Orem C, Baykan M. Aortic pulse wave velocity in subjects with aortic valve sclerosis. *Echocardiography*. 2008;25:1112-1116
147. Walsh TF, Dall'Armellina E, Chughtai H, Morgan TM, Ntim W, Link KM, Hamilton CA, Kitzman DW, Hundley WG. Adverse effect of increased left ventricular wall thickness on five year outcomes of patients with negative dobutamine stress. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2009;11:25
148. Verdecchia P, Schillaci G, Borgioni C, Ciucci A, Battistelli M, Bartoccini C, Santucci A, Santucci C, Reboldi G, Porcellati C. Adverse prognostic significance of concentric remodeling of the left ventricle in hypertensive patients with normal left ventricular mass. *Journal of the American College of Cardiology*. 1995;25:871-878
149. Schillaci G, Verdecchia P, Porcellati C, Cuccurullo O, Cosco C, Perticone F. Continuous relation between left ventricular mass and cardiovascular risk in essential hypertension. *Hypertension*. 2000;35:580-586
150. Huang BT, Peng Y, Liu W, Zhang C, Huang FY, Wang PJ, Zuo ZL, Liao YB, Chai H, Huang KS, Huang DJ, Chen M. Increased interventricular septum wall thickness predicts all-cause death in patients with coronary artery disease. *Internal medicine journal*. 2015;45:275-283

151. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Left ventricular mass and incidence of coronary heart disease in an elderly cohort. The framingham heart study. *Annals of internal medicine*. 1989;110:101-107
152. Reckelhoff JF. Gender differences in the regulation of blood pressure. *Hypertension*. 2001;37:1199-1208
153. Aronow WS, Ahn C, Shirani J, Kronzon I. Comparison of frequency of new coronary events in older subjects with and without valvular aortic sclerosis. *The American journal of cardiology*. 1999;83:599-600, A598
154. Rieck AE, Gerdts E, Lonnebakken MT, Bahlmann E, Cioffi G, Gohlke-Barwolf C, Ray S, Cramariuc D. Global left ventricular load in asymptomatic aortic stenosis: Covariates and prognostic implication (the seas trial). *Cardiovascular ultrasound*. 2012;10:43
155. Hachicha Z, Dumesnil JG, Pibarot P. Usefulness of the valvuloarterial impedance to predict adverse outcome in asymptomatic aortic stenosis. *Journal of the American College of Cardiology*. 2009;54:1003-1011
156. Laskey WK, Kussmaul WG. Pressure recovery in aortic valve stenosis. *Circulation*. 1994;89:116-121
157. Herment A, Kachenoura N, Lefort M, Bensalah M, Dogui A, Frouin F, Mousseaux E, De Cesare A. Automated segmentation of the aorta from phase contrast mr images: Validation against expert tracing in healthy volunteers and in patients with a dilated aorta. *Journal of magnetic resonance imaging : JMRI*. 2010;31:881-888
158. Fantin F, Mattocks A, Bulpitt CJ, Banya W, Rajkumar C. Is augmentation index a good measure of vascular stiffness in the elderly? *Age and ageing*. 2007;36:43-48
159. Takase H, Dohi Y, Kimura G. Distribution of central blood pressure values estimated by omron hem-9000ai in the japanese general population. *Hypertension research : official journal of the Japanese Society of Hypertension*. 2013;36:50-57
160. Herbert A, Cruickshank JK, Laurent S, Boutouyrie P, Reference Values for Arterial Measurements C. Establishing reference values for central blood pressure and its amplification in a general healthy population and according to cardiovascular risk factors. *European heart journal*. 2014;35:3122-3133
161. Burk J, Blanke P, Stankovic Z, Barker A, Russe M, Geiger J, Frydrychowicz A, Langer M, Markl M. Evaluation of 3d blood flow patterns and wall shear stress in the normal and dilated thoracic aorta using flow-sensitive 4d cmr. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2012;14:84
162. Hess AT BM, Glaze SJ, Pitcher A, Myerson S, Neubauer S, et al. Evaluation of circulation, γ, as a quantifying metric in 4d flow mri. . *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2013;15:E36
163. Rabkin SW. Differential expression of mmp-2, mmp-9 and timp proteins in thoracic aortic aneurysm - comparison with and without bicuspid aortic valve: A meta-analysis. *VASA. Zeitschrift fur Gefasskrankheiten*. 2014;43:433-442
164. Tadros TM, Klein MD, Shapira OM. Ascending aortic dilatation associated with bicuspid aortic valve: Pathophysiology, molecular biology, and clinical implications. *Circulation*. 2009;119:880-890
165. Bieging ET, Frydrychowicz A, Wentland A, Landgraf BR, Johnson KM, Wieben O, Francois CJ. In vivo three-dimensional mr wall shear stress estimation in ascending aortic dilatation. *Journal of magnetic resonance imaging : JMRI*. 2011;33:589-597
166. Vahanian A, Baumgartner H, Bax J, Butchart E, Dion R, Filippatos G, Flachskampf F, Hall R, Iung B, Kasprzak J, Nataf P, Tornos P, Torracca L, Wenink A, Task Force on the Management of Valvular Hearth Disease of the European Society of C, Guidelines ESCCfP. Guidelines on the management of valvular heart disease: The task force on the management of valvular heart disease of the european society of cardiology. *European heart journal*. 2007;28:230-268

167. Hess OM, Ritter M, Schneider J, Grimm J, Turina M, Krayenbuehl HP. Diastolic function in aortic valve disease: Techniques of evaluation and pre-/postoperative changes. *Herz*. 1984;9:288-296
168. Kupari M, Turto H, Lommi J. Left ventricular hypertrophy in aortic valve stenosis: Preventive or promotive of systolic dysfunction and heart failure? *European heart journal*. 2005;26:1790-1796
169. Myerson SG, Bellenger NG, Pennell DJ. Assessment of left ventricular mass by cardiovascular magnetic resonance. *Hypertension*. 2002;39:750-755
170. Hor KN, Gottliebson WM, Carson C, Wash E, Cnota J, Fleck R, Wansapura J, Klimeczek P, Al-Khalidi HR, Chung ES, Benson DW, Mazur W. Comparison of magnetic resonance feature tracking for strain calculation with harmonic phase imaging analysis. *JACC. Cardiovascular imaging*. 2010;3:144-151
171. Dahl JS, Videbaek L, Poulsen MK, Rudbaek TR, Pellikka PA, Moller JE. Global strain in severe aortic valve stenosis: Relation to clinical outcome after aortic valve replacement. *Circulation. Cardiovascular imaging*. 2012;5:613-620
172. Nagel E, Stuber M, Burkhard B, Fischer SE, Scheidegger MB, Boesiger P, Hess OM. Cardiac rotation and relaxation in patients with aortic valve stenosis. *European heart journal*. 2000;21:582-589
173. Grayburn PA, Appleton CP, DeMaria AN, Greenberg B, Lowes B, Oh J, Plehn JF, Rahko P, St John Sutton M, Eichhorn EJ, Investigators BTES. Echocardiographic predictors of morbidity and mortality in patients with advanced heart failure: The beta-blocker evaluation of survival trial (best). *Journal of the American College of Cardiology*. 2005;45:1064-1071
174. Gotte MJ, Germans T, Russel IK, Zwanenburg JJ, Marcus JT, van Rossum AC, van Veldhuisen DJ. Myocardial strain and torsion quantified by cardiovascular magnetic resonance tissue tagging: Studies in normal and impaired left ventricular function. *Journal of the American College of Cardiology*. 2006;48:2002-2011
175. Shehata ML, Cheng S, Osman NF, Bluemke DA, Lima JA. Myocardial tissue tagging with cardiovascular magnetic resonance. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2009;11:55
176. Taylor RJ, Moody WE, Umar F, Edwards NC, Taylor TJ, Stegemann B, Townend JN, Hor KN, Steeds RP, Mazur W, Leyva F. Myocardial strain measurement with feature-tracking cardiovascular magnetic resonance: Normal values. *European heart journal cardiovascular Imaging*. 2015;16:871-881
177. Nightingale AK, Sverdlov AL, Rajendran S, Mishra K, Heresztyn T, Ngo DT, Horowitz JD. Lack of association between aortic sclerosis and left ventricular hypertrophy in elderly subjects. *International journal of cardiology*. 2011;150:33-38
178. Demir M. Left ventricular systolic and diastolic function in subjects with a bicuspid aortic valve without significant valvular dysfunction. *Experimental and clinical cardiology*. 2013;18:e1-4
179. Galanti G, Stefani L, Toncelli L, Vono MC, Mercuri R, Maffulli N. Effects of sports activity in athletes with bicuspid aortic valve and mild aortic regurgitation. *British journal of sports medicine*. 2010;44:275-279
180. Santarpia G, Scognamiglio G, Di Salvo G, D'Alto M, Sarubbi B, Romeo E, Indolfi C, Cotrufo M, Calabro R. Aortic and left ventricular remodeling in patients with bicuspid aortic valve without significant valvular dysfunction: A prospective study. *International journal of cardiology*. 2012;158:347-352
181. Bilen E, Akcay M, Bayram NA, Kocak U, Kurt M, Tanboga IH, Bozkurt E. Aortic elastic properties and left ventricular diastolic function in patients with isolated bicuspid aortic valve. *The Journal of heart valve disease*. 2012;21:189-194
182. Augustine D, Lewandowski AJ, Lazdam M, Rai A, Francis J, Myerson S, Noble A, Becher H, Neubauer S, Petersen SE, Leeson P. Global and regional left ventricular myocardial deformation measures by magnetic resonance feature tracking in healthy volunteers:

Comparison with tagging and relevance of gender. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2013;15:8
183. Becker M, Kramann R, Dohmen G, Luckhoff A, Autschbach R, Kelm M, Hoffmann R. Impact of left ventricular loading conditions on myocardial deformation parameters: Analysis of early and late changes of myocardial deformation parameters after aortic valve replacement. *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography*. 2007;20:681-689
184. Orwat S, Kempny A, Diller GP, Bauerschmitz P, Bunck A, Maintz D, Radke RM, Baumgartner H. Cardiac magnetic resonance feature tracking: A novel method to assess myocardial strain. Comparison with echocardiographic speckle tracking in healthy volunteers and in patients with left ventricular hypertrophy. *Kardiologia polska*. 2014;72:363-371
185. Kilner PJ, Yang GZ, Wilkes AJ, Mohiaddin RH, Firmin DN, Yacoub MH. Asymmetric redirection of flow through the heart. *Nature*. 2000;404:759-761
186. Carlsson M, Heiberg E, Toger J, Arheden H. Quantification of left and right ventricular kinetic energy using four-dimensional intracardiac magnetic resonance imaging flow measurements. *American journal of physiology. Heart and circulatory physiology*. 2012;302:H893-900
187. Stoll V HA, Eriksson J, Dyverfeldt P, Ebbers T, Myerson S, Carlhall C, Neubauer S. The kinetic energies of left ventricular 4d flow components correlate with established markers of prognosis and represent novel imaging biomarkers in both ischaemic and dilated cardiomyopathy. *Journal of Cardiovascular Magnetic Resonance*. 2016;18
188. Iung B, Baron G, Butchart EG, Delahaye F, Gohlke-Barwolf C, Levang OW, Tornos P, Vanoverschelde JL, Vermeer F, Boersma E, Ravaud P, Vahanian A. A prospective survey of patients with valvular heart disease in europe: The euro heart survey on valvular heart disease. *European heart journal*. 2003;24:1231-1243
189. Ross J, Jr., Braunwald E. Aortic stenosis. *Circulation*. 1968;38:61-67
190. Amato MC, Moffa PJ, Werner KE, Ramires JA. Treatment decision in asymptomatic aortic valve stenosis: Role of exercise testing. *Heart*. 2001;86:381-386
191. Marechaux S, Hachicha Z, Bellouin A, Dumesnil JG, Meimoun P, Pasquet A, Bergeron S, Arsenault M, Le Tourneau T, Ennezat PV, Pibarot P. Usefulness of exercise-stress echocardiography for risk stratification of true asymptomatic patients with aortic valve stenosis. *European heart journal*. 2010;31:1390-1397
192. Francis DP, Shamim W, Davies LC, Piepoli MF, Ponikowski P, Anker SD, Coats AJ. Cardiopulmonary exercise testing for prognosis in chronic heart failure: Continuous and independent prognostic value from ve/vco(2)slope and peak vo(2). *European heart journal*. 2000;21:154-161
193. Steadman CD, Jerosch-Herold M, Grundy B, Rafelt S, Ng LL, Squire IB, Samani NJ, McCann GP. Determinants and functional significance of myocardial perfusion reserve in severe aortic stenosis. *JACC. Cardiovascular imaging*. 2012;5:182-189
194. Rajani R, Rimington H, Chambers JB. Treadmill exercise in apparently asymptomatic patients with moderate or severe aortic stenosis: Relationship between cardiac index and revealed symptoms. *Heart*. 2010;96:689-695
195. Das P, Rimington H, Chambers J. Exercise testing to stratify risk in aortic stenosis. *European heart journal*. 2005;26:1309-1313
196. Buchfuhrer MJ, Hansen JE, Robinson TE, Sue DY, Wasserman K, Whipp BJ. Optimizing the exercise protocol for cardiopulmonary assessment. *Journal of applied physiology: respiratory, environmental and exercise physiology*. 1983;55:1558-1564
197. Naughton J, Sevelius G, Balke B. Physiological responses of normal and pathological subjects to a modified work capacity test. *The Journal of sports medicine and physical fitness*. 1963;3:201-207

198. Older P, Hall A, Hader R. Cardiopulmonary exercise testing as a screening test for perioperative management of major surgery in the elderly. *Chest*. 1999;116:355-362
199. Smith TB, Stonell C, Purkayastha S, Paraskevas P. Cardiopulmonary exercise testing as a risk assessment method in non cardio-pulmonary surgery: A systematic review. *Anaesthesia*. 2009;64:883-893
200. Grant SW, Hickey GL, Wisely NA, Carlson ED, Hartley RA, Pichel AC, Atkinson D, McCollum CN. Cardiopulmonary exercise testing and survival after elective abdominal aortic aneurysm repairdagger. *British journal of anaesthesia*. 2015;114:430-436