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Subclinical Markers of Disease Burden of Severe Aortic Stenosis in Patients with Type 2 Diabetes Mellitus and the Metabolic Syndrome

by

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Thesis for the degree of Doctor of Medicine

April 2024

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF MEDICINE

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SUBCLINICAL MARKERS OF DISEASE BURDEN OF SEVERE AORTIC STENOSIS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND THE METABOLIC SYNDROME

Suresh Giritharan

Background: Aortic valve stenosis (AS) is the most common valvular disease in the developed world with a global prevalence of around 2-9% in people over the age of 65. Patients with both Type 2 diabetes mellitus (T2DM) and metabolic syndrome (MetS) have been reported to have worse outcomes following aortic valve replacement surgery (AVR). Mechanisms leading to aortic valve calcification and left ventricular changes in AS patients are distinct from coronary calcification. This study aimed to identify preoperative and postoperative differences in the ventricular function of patients with severe AS undergoing AVR, and to assess the preoperative levels of selected biomarkers of lipid metabolism in three different subsets of people from a separate cohort.

Methods: This thesis is comprised of two separate study cohorts. The first study involved a retrospective cohort of 367 people who underwent isolated aortic valve replacement at a secondary care cardiothoracic surgery unit in Wessex. They were subdivided into three groups; people without T2DM or MetS, people with MetS, and people with diabetes. Alongside baseline demographic and biochemical data, preoperative transthoracic

parameters were collated. This was compared with 1-year postoperative transthoracic echocardiography. Changes in ventricular dimensions and mass were interrogated.

The second study involved a separate, prospective cohort of forty-two participants, who were also subdivided into the same three groupings as the first study. The same demographic, biochemical and echocardiographic data was once again collated. In addition to this, serum samples were collected to test for six biomarkers of lipid metabolism.

Results: The first study noted that patients with MetS and T2DM had more severe left ventricular remodelling preoperatively. Postoperatively however, these same participants experienced less reverse remodelling (a beneficial sequelae of aortic valve replacement) than patients without T2DM or MetS. The second study, which focused on adipokine and lipoprotein profiles, demonstrated that people with T2DM and MetS had increased levels of resistin, lipoprotein-A and apolipoprotein-B1 compared to the control group. The control group, on the other hand, had increased levels of adiponectin and leptin compared to the other two groups. Although variations in these markers were distinct, no direct correlation with preoperative echocardiographic findings was demonstrated.

Conclusion: This thesis concludes that in essence, MetS and T2DM patients with similar presentations of severe AS have significantly worse subclinical myocardial changes. The observed differences in the levels of adiponectin, leptin, resistin, lipoprotein-A and apolipoprotein-B1 adds to the current knowledge base and ongoing understanding of these biomarkers in specific cohorts of people with MetS, T2DM and symptomatic AS.

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Declaration of Authorship

I, Suresh Giritharan declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

SUBCLINICAL MARKERS OF DISEASE BURDEN OF SEVERE AORTIC STENOSIS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND THE METABOLIC SYNDROME

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University.
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated.
3. Where I have consulted the published work of others, this is always clearly attributed.
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
5. I have acknowledged all main sources of help.
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

Signed: Date: 01/10/2022

Acknowledgements

I would like to acknowledge the support of Kerry Hyde and Melissa Doherty (University of Southampton) for their teaching and support in the laboratory. Dr. Susan Wilson for her guidance in methodology at the conception of this project in the Histology Research Unit (University of Southampton).

A special mention goes to cardiothoracic surgeons Mr. Kareem Salhiyyah and Mr. Simon Duggan (University Hospital Southampton) who were a constant source of advice and support in the initial stages of my research journey.

This thesis would not have been possible without the guidance and support from the supervisory team comprising of Dr. Felino Cagampang and Dr. Christopher Torrens (University of Southampton), and Professor Sunil Ohri (Consultant Cardiac Surgeon, University Hospital Southampton). I am honoured to have my project overseen my such a knowledgeable, experienced, and dedicated team.

To Sharmila, Vikram and Anusha - who make it all worthwhile

Definitions and Abbreviations

ANCOVA	Analysis of Covariance
ANOVA	One-way Analysis of Variance
AF	Atrial Fibrillation
Apo A1	Apolipoprotein A1
Apo B	Apolipoprotein B
AS	Aortic Stenosis
AU	Agatston Units
AVA	Aortic Valve Area
AVR	Aortic Valve Replacement
BMI	Body Mass Index
BSA	Body Surface Area
CAD	Coronary Artery Disease
CAVD	Calcific Aortic Valve Disease
CCS	Canadian Cardiovascular Society Angina Score
CMR	Cardiac Magnetic Resonance imaging
CO	Cardiac Output
CRP	C-Reactive Protein
CT	Computed Tomography
EACTS	European Association of Cardiothoracic Surgery
ECG	Electrocardiogram
ECM	Extracellular matrix
EDV	End-diastolic volume
ESC	European Society of Cardiology
ESV	End-systolic volume
EuroSCORE	European System for Cardiac Operative Risk Evaluation
HbA1C	Glycated haemoglobin A1C
HDL	High-density lipoprotein
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A reductase

IVS	Interventricular septal thickness
LAD	Left atrial diameter
LDL	Low density lipoprotein
Lp(a)	Lipoprotein A
LV	Left Ventricle
LVEDD	Left Ventricular end-diastolic diameter
LVESD	Left Ventricular end-systolic diameter
LVEF	Left Ventricular Ejection Fraction
LVH	Left Ventricular Hypertrophy
LVM	Left Ventricular Mass
LVMi	Left Ventricular Mass index
LVOT	Left Ventricular Outflow Tract
MACCE	Major Adverse Cardiovascular and Cerebrovascular Events
MetS	Metabolic Syndrome
MPG	Mean pressure gradient
MR	Mitral Valve Regurgitation
NCEP: ATP III	National Cholesterol Education Program's Adult Treatment Panel III
NLR	Neutrophil to lymphocyte ratio
NYHA	New York Heart Association Functional Classification
PLAX	Parasternal Long Axis View
PLR	Platelet to Lymphocyte ratio
PSAX	Parasternal Short Axis VIew
PV	Pulmonary Valve
PWT	Posterior Wall Thickness
RA	Right Atrium
RVOT	Right Ventricular Outflow Tract
RWMA	Regional Wall Motion Abnormality
RWT	Relative Wall Thickness ratio
SAVR	Surgical Aortic Valve Replacement
SD	Standard Deviation
SLRP	Small leucine-rich proteoglycans

STS	Society for Thoracic Surgeons
SVR	Systemic Vascular Resistance
TAVI	Transcatheter Aortic Valve Implantation
TTE	Transthoracic Echocardiography
TV	Tricuspid Valve
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
WHO	World Health Organization

Declaration of Published Material

Parts of this work have been published as below:

Giritharan S, Cagampang F, Torrens C, Salhiyyah K, Duggan S, Ohri S Aortic Stenosis Prognostication in Patients With Type 2 Diabetes: Protocol for Testing and Validation of a Biomarker-Derived Scoring System JMIR Res Protoc 2019;8(8):e13186 DOI: 10.2196/13186 PMID: 31407670 PMCID: 6818526

Chapter 1. Introduction and Review of Literature

Type 2 diabetes mellitus (T2DM) and aortic stenosis (AS) frequently coexist in the adult population, with both these diseases following a progressive path with advancing age (1). Current evidence has demonstrated that T2DM has a perpetuating effect on the underlying cellular and molecular mechanisms which ultimately lead to AS, namely chronic inflammation, increased osteoblastic activity, interstitial fibrosis, oxidative stress, lipid deposition and active deposition of calcium on valve leaflets (2). These, in turn, result in the characteristic narrowing of the valve orifice that compromises the optimal delivery of oxygenated blood to organs. Patients with T2DM tend to present with symptomatic AS at a younger age and experience higher rates of morbidity and mortality than patients without T2DM (3). This is reflected in poor prognostication of patients with T2DM undergoing aortic valve replacement surgery (AVR) in both the European System for Cardiac Operative Risk Evaluation (EuroSCORE) and Society of Thoracic Surgeons (STS) risk stratification scoring systems (4,5). Alongside advancing age, risk factors such as hypertension, dyslipidaemia, obesity, smoking and renal dysfunction contribute to AS (6). As life expectancy in high-income nations continues to rise, it is reasonable to expect that the prevalence of patients with AS and T2DM will rise correspondingly, therefore necessitating comprehensive management and preventative strategies for this complex cohort of patients.

Henceforth, the current understanding and evidence of the significance of T2DM in the development and progression of degenerative AS will be discussed, both in terms of valvular insult as well as in terms of functional changes of the left ventricle (LV). Emphasis will be placed on establishing common factors which contribute to the shared aetiological underpinnings of T2DM and AS and reviewing methods of better monitoring of disease progression to guide the timing of intervention by valve replacement in an effort to improve both organ-specific outcomes as well as reduce the risk of Major Adverse Cardiovascular and Cerebrovascular Events (MACCE). The shared cellular and molecular mechanisms involved, in particular lipid pathways, will also be discussed to justify the

need for focused study of selected pathway elements and assess their feasibility as biomarkers or functional markers of prognostication in early stages of this disease, the modulation of which could form the basis of targeted medical therapy in the future.

1.1 Overview of AS

Aortic stenosis (AS) is the pathological narrowing of the aortic valve of the heart (see Figure 1), resulting in increased resistance to the flow of blood leaving the heart and entering the systemic circulation (7). More than one in eight people aged 75 and older have moderate or severe aortic stenosis (8). The most common cause in the developed world is an age-associated degenerative process termed Calcific Aortic Valve Disease (CAVD), with an estimated prevalence of 2-9% in adults over 65 years old (9). In younger patients, the cause is most commonly due to a congenital bicuspid aortic valve, a structure that is usually composed of three leaflets. The prevalence of bicuspid aortic valve is 0.5-1% in children (10). Rheumatic heart disease also results in aortic stenosis, however, the incidence and prevalence of this inflammatory disease are declining in the developed world (11).

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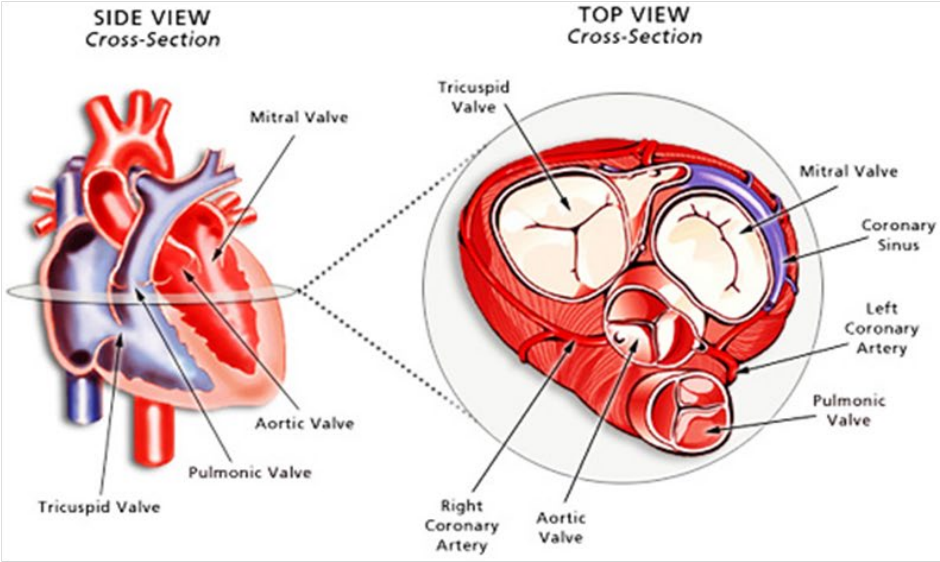

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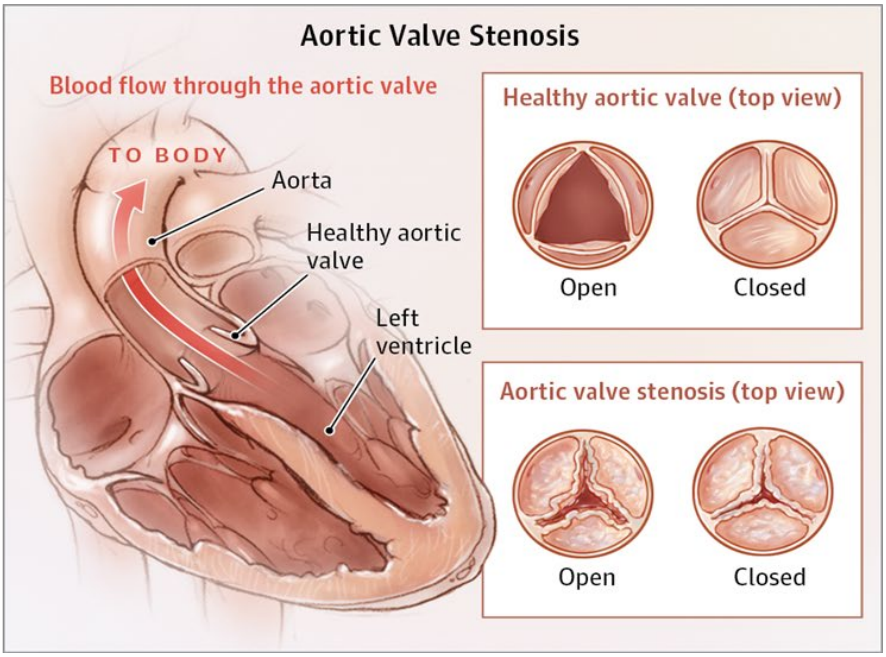


Figure 1. (**a**) Position of the aortic valve in relation to the cardiac chambers (a), coronary arteries and other valves. Blue and red hues denote deoxygenated and oxygenated blood respectively. Image credit; Urman MK and Caren JF. (**b**) Image on the upper right demonstrates the normal opening of the aortic valve. Calcification of the valve leaflets reduced pliability, resulting in impaired valve opening (bottom right) when blood is ejected from the left ventricle during ventricular contraction. Image credit; Patel A et al. (12)

1.2 Pathophysiology of AS

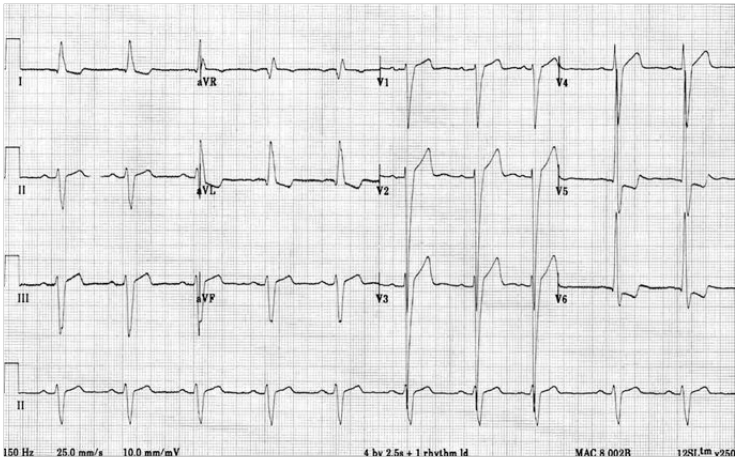
The narrowing of the valve in aortic stenosis is the result of the thickening of the individual leaflets (See Figure 1b), rendering them unable to open fully in ventricular systole (contraction of the ventricle causing ejection of blood into the ascending aorta and systemic circulation). The immediate consequence of this is that the physiological demands for oxygenated haemoglobin from blood necessary for the metabolic demands and optimal functioning of various organs cannot be effectively met, particularly during periods of increased sympathetic nervous stimulation such as physical exercise, exposure to cold temperatures or emotional stress. Suboptimal blood flow to the coronary and carotid arterial systems, and back-pressure of blood into the pulmonary vasculature (due to increased left ventricular end diastolic pressure) are responsible for the classical symptoms of aortic stenosis which are chest pain, dizziness, and shortness of breath respectively. In severe cases, collapse and even sudden death can occur. Long-term consequences of aortic stenosis stem from the incomplete ejection of blood from the left ventricle (13). The increase in blood volume in the left ventricle over time results in enlargement (hypertrophy) of the ventricle. This enlargement may be due to increasing thickness of the ventricular wall (concentric hypertrophy) or laxity of the normally elastic ventricular myocardium (eccentric hypertrophy) – both these forms of hypertrophy are less efficient in ejecting blood. Over time, this results in left ventricular failure due to back-pressure which is transmitted retrograde through the left atrium (causing dilatation and atrial fibrillation) and pulmonary circulation (causing pulmonary oedema).

1.3 Diagnosis, Imaging and Management of AS

AS should be considered when patients present with the triad of symptoms mentioned above, however incidental findings of an ejection systolic murmur on precordial auscultation or evidence of left ventricular hypertrophy (LVH) on electrocardiography (ECG) are common (Figure 2a). The initial screening ventriculogram (injection of contrast into the left ventricle during coronary angiography)

may demonstrate braking (i.e. compromise of the usual laminar flow of a uniform column of blood) of ejection of blood from the LV into the ascending aorta (Figure 2b).

a.



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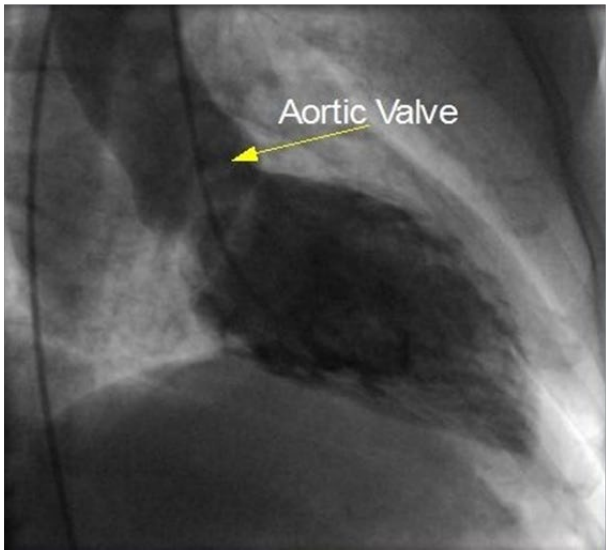


Figure 2. (**a**) Standard 12-lead echocardiogram (ECG) demonstrating left ventricular hypertrophy (LVH). (**b**) Right anterior oblique (RAO) view of ventriculogram performed during cardiac catheterization for angiography. A dynamic view would qualitatively demonstrate any impairment to ejection of blood from the left ventricle during systole (contraction) into the ascending aorta. Image credit; author's own.

All of these patients will undergo transthoracic echocardiography (TTE – ultrasonic imaging of the heart for both structural statuses and to assess dynamic performance, see Figure 3a & 3B) for the comprehensive evaluation of the diseased valve and to assess ventricular function (14). Standard parameters for assessment of aortic stenosis as per European Society of Echocardiography (ESC) guidelines are aortic jet velocity (m/s), mean transvalvular gradient (mmHg), aortic valve area (AVA) (cm^2) and indexed AVA (cm^2/m^2) (see Table 1). LV ejection fraction (LVEF), LV systolic and diastolic dysfunction and regional wall motion abnormalities (RWMA) provide information on the status of the myocardium, guiding preoperative or supportive pharmacotherapy and allows for planning of operative strategy. Imaging by computed tomography (CT) provides a different dimension to valve assessment (15). Although the effect of haemodynamic compromise is not assessed as it is in TTE, this static imaging modality provides a more accurate assessment of the calcific burden of the valve and better delineation of anatomy, both of which aid in guiding interventional strategies.

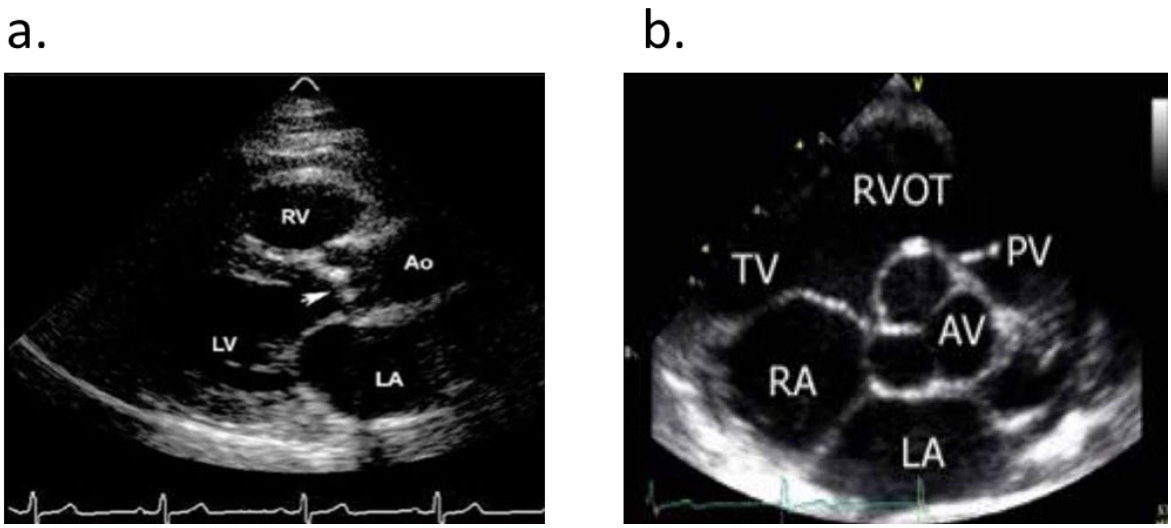


Figure 3. (**a**) Transthoracic echocardiogram (TTE) image in parasternal long axis (PLAX) view of the heart in diastole (relaxation) demonstrating a stenotic aortic valve (arrow), characterised by increased echogenicity and suboptimal closure. LV; left ventricle, RV; right ventricle, Ao; ascending aorta, LA; left atrium. (**b**) TTE image in parasternal short axis view (PSAX) demonstrating normal closure of a trileaflet aortic valve (AV) in which all three valve tips are of normal thickness and converge at the centre point. RVOT; right ventricular outflow tract, TV; tricuspid valve, PV; pulmonary valve, RA; right atrium, LA; left atrium. Image credit; author's own.

Table 1. Diagnostic criteria for AS by Transthoracic echocardiographic (TTE) imaging. Image credit; Carabello et al. (16)

Severity	Valve Area (cm^2)	Mean Gradient (mmHg)	Velocity (m/s)	Indexed Valve Area (cm^2/m^2)
Mild	>1.5	<20	2.6-2.9	>0.85
Moderate	1.0-1.5	20-40	3.0-4.0	0.60-0.85
Severe	<1.0	>40	>4.0	<0.6
Critical	<0.5	--	--	--

As there is currently no clinically effective pharmacological therapy that can prevent the onset, progression or regression of aortic stenosis, treatment consists of the replacement of the aortic valve. This is part of the decision tree in the management of AS (Figure 4).

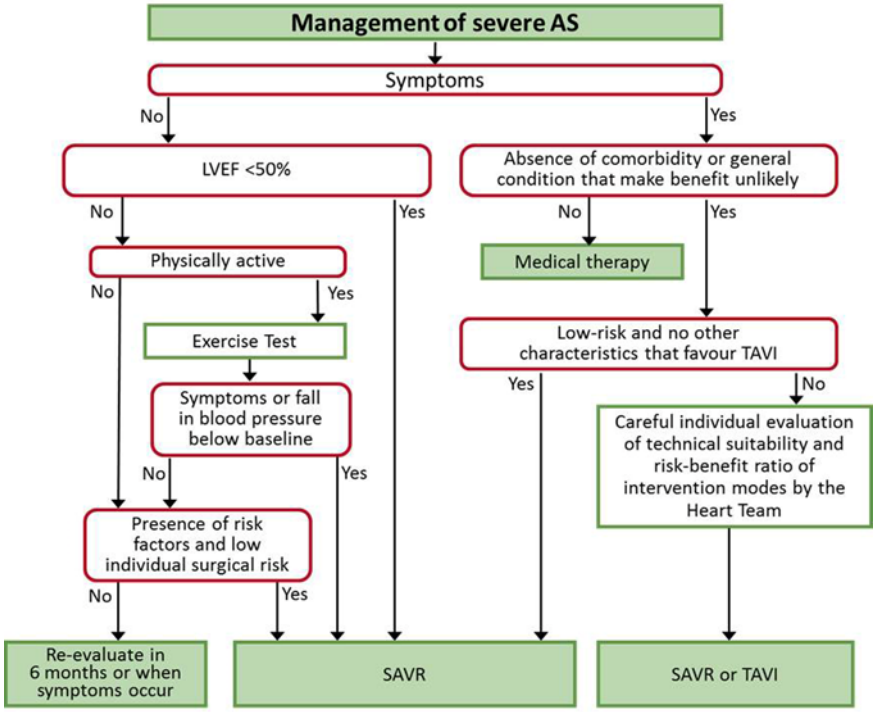
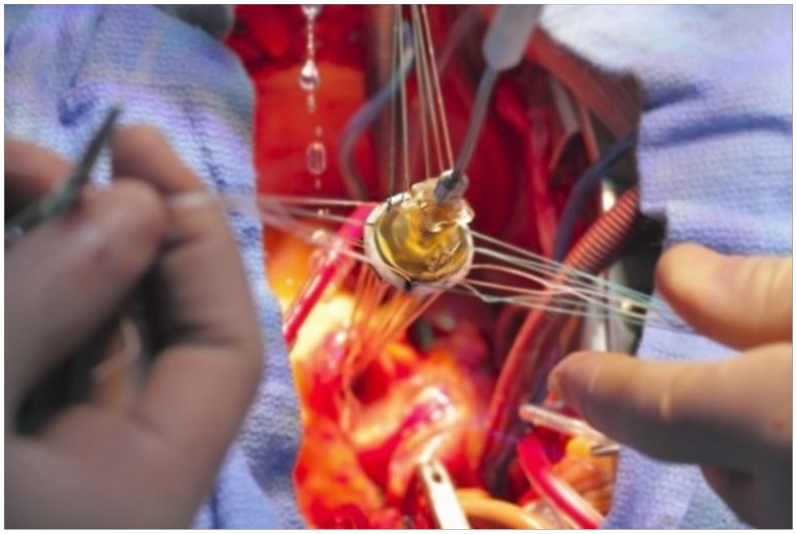


Figure 4. Decision tree for the management of severe aortic stenosis. AS, aortic stenosis; LVEF, left ventricular ejection fraction; TAVI, transcatheter aortic valve implantation; SAVR, surgical aortic valve replacement. Image credit; The Task Force for the Management of Valvular Heart Disease of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS) (20)

Surgical aortic valve replacement (AVR) has long been the gold standard treatment (Figure 5a), with its origins dating back to Dwight E. Harken's successful pioneering implantation of a ball-caged valve device in a patient in 1960 (17). Today, aortic valve implants can be in the form of either a tissue bioprosthesis – constructed using bovine or porcine pericardial tissue – or a mechanical prosthesis made with various combinations of pyrolytic carbon, titanium, polytetrafluoroethylene, polyester and Dacron (18). The last decade has seen major advancements in transcatheter aortic valve implantation (TAVI) (Figure 5b) – a procedure where a prosthetic valve is inserted via a large artery (usually the femoral or subclavian artery) under radiological guidance (19). The prosthetic valve is deployed within the native diseased valve (splinting it open against the inner wall of the aortic root), thus commandeering the role of the native aortic valve.

a.



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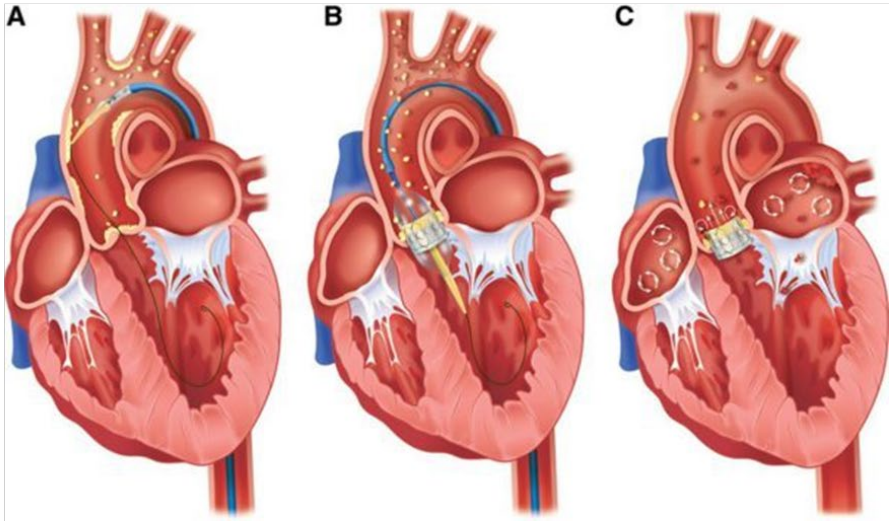


Figure 5. (**a**) Surgical aortic valve replacement (AVR). The patient's head is towards the bottom of the picture. The anaesthetized patient is connected to a cardiopulmonary bypass (heart-lung) machine via large-bore cannulae in the right atrium and ascending aorta and a cross-clamp is placed beneath the aortic cannula. The heart is then arrested using a potassium-rich solution. This allows for a still, bloodless field facilitating surgery. (**b**) Diagram illustrating transcatheter aortic valve implantation (TAVI), performed under radiological guidance. A guidewire is inserted via the femoral artery and threaded into the left ventricle through the aortic valve orifice (A). A catheter containing a remotely-inflatable balloon and a prosthetic aortic valve (in collapsed form) is positioned at the aortic valve annulus. The balloon is then inflated, causing the new valve to expand and fracture the native valve. The splinting of the native valve against the aortic wall also provides a point of traction for the new valve thereby preventing dislodgement.

1.4 Parallels and divergences between AS and atherosclerosis

Historical observations of the prevalence of calcific aortic valve disease in the elderly population may have been responsible for the misnomer of "degenerative" aortic stenosis. Until the beginning of the 21st century, the theory of passive wear-and-tear of the endothelial surface of the aortic valve leaflets due to the sustained increase in blood pressure and turbulent flow was widely accepted (21). Numerous studies of valvular histopathology and clinical data over the last two decades have demonstrated that aortic valve calcification is an active process which shares similarities with atherosclerosis, as AS frequently coexisted alongside arterial disease, namely coronary artery disease (CAD) (22). Molecular changes such as osteogenic metaplasia, chronic inflammation, lipoprotein deposition and oxidative stress culminate in two distinct but intertwined processes that are responsible for the narrowing of the valve; fibrosis and calcification (23). Despite these apparent parallels in pathogenesis, studies have demonstrated that the same pharmacological targets for lipid-lowering therapy that is inhibited in coronary and peripheral artery disease such as 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA, targeted by statins) and the sterol transporter Niemann-Pick C1-like 1 (targeted by ezetimibe) have failed to show retardation in disease progression or demonstrate a reduction in major cardiovascular and cerebrovascular events of morbidity and mortality (MACCE) in symptomatic aortic stenosis as they do in atherosclerotic disease (24).

Data from large clinical cohorts also attest to this link between AS and atherosclerosis. A shared risk factor profile consisting of increased age, male gender, increased low-density lipoprotein (LDL) levels, hypertension, increased body mass index (BMI) smoking, diabetes mellitus and chronic renal dysfunction allude to common aetiological origins (25). Furthermore, coronary artery disease, T2DM and AS are frequently seen to be prevalent in the ageing Western population and treated concomitantly, and the risk of surgery in these patients is much increased when calculated using the scoring systems mentioned previously.

1.5 Diabetes Mellitus

Diabetes mellitus is a metabolic disease, characterized by chronic elevation of glucose levels in plasma. This condition covers a broad range of subcategories such as type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and other entities such as sequelae of other endocrine conditions (26). This thesis will focus on the two main subtypes of diabetes mellitus, namely T1DM and T2DM. Both of these subtypes are the end result of either defective insulin secretion (insulin deficiency), the impaired uptake and action of insulin (insulin resistance), or both (27).

Insulin, a peptide hormone secreted by the beta cells of the pancreatic islets of Langerhans, plays a central role in mediating the uptake of glucose into cells of various organs which is essential for metabolic functions (28). The eventual end result in long-term dysregulation of optimal cellular glucose uptake is a myriad of complications, particularly of the cardiovascular system. Every week in the United Kingdom, more than 770 strokes, 590 heart attacks, 184 limb amputations and 2300 new diagnoses of heart failure is attributed to diabetes mellitus (29).

1.5.1 Prevalence of Diabetes in the United Kingdom

The most recent figures provided by Diabetes UK show that the current prevalence of diabetes in the United Kingdom is 4.3 million at the end of the year 2022 with an estimate of approximately 850,000 undiagnosed people, bringing the estimated total to above 5 million people. Registration of new patients with diabetes, a proxy for incidence, has increased for the year ending 2022 by 148,951 patients compared to the previous year. This is considerably higher than the previous incidence figure of 3.7 million people at the end of the year 2017. Diabetes UK also estimates that a further 2.4 million people are at high risk of developing T2DM. Approximately 90% of these people have a diagnosis of

T2DM, and 8% have a diagnosis of T1DM. The remaining 2% encapsulates all the other subtypes of diabetes, including the ones mentioned in the paragraph above (30).

1.5.2 Overview of Type 1 and Type 2 Diabetes Mellitus

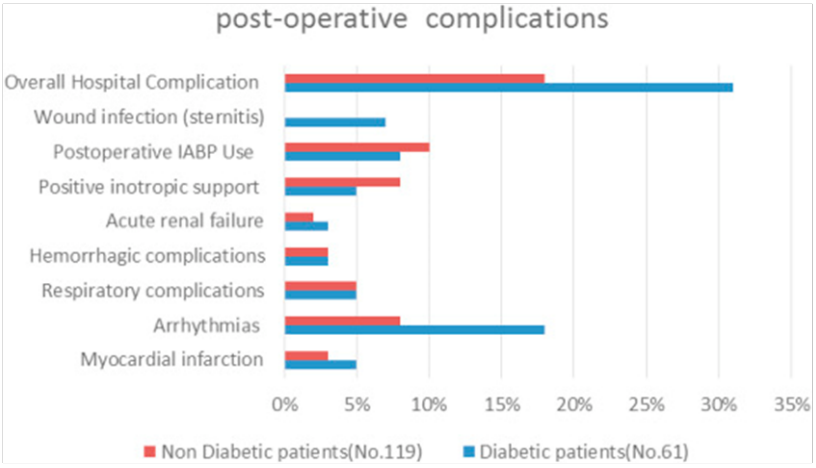
Type 1 diabetes mellitus is the result of an autoimmune response to proteins of the pancreatic islet cells. Alongside the decreased insulin secretion secondary to pancreatic beta cell destruction, there is also deranged pancreatic alpha cell function which results in excessive secretion of the hormone glucagon, which is paradoxical compared to hyperglycaemia which is not associate with T1DM. This has deleterious metabolic effects such as the rapid development of diabetic ketoacidosis (DKA) which may occur if people with T1DM do not receive timely insulin therapy. Although T1DM may be diagnosed at any age, the peak presentation is around the time of puberty (31).

Type 2 diabetes mellitus is a heterogenous condition that results from a combination of genetic factors (ultimately leading to insulin resistance and secretion) as well as environmental and lifestyle factors. Increased incidences are seen in higher-income countries. Factors such as a lack of physical activity, a food environment consisting of more heavily-processed calorie-dense products, over-eating, stress and advancing age have all been confirmed as contributing factors. These factors have subsequently formed the basis of public health policy in most developed and developing countries. People are typically diagnosed with T2DM between the fourth and sixth decades of life, although the increasing incidence of childhood obesity has resulted in increasing presentations earlier than this. Patients of Afro-Caribbean and South Asia ethnic backgrounds generally present slightly earlier than their Caucasian counterparts, however it remains inconclusive as to whether this effect is genetically or environmentally driven (32).

1.5.3 Type 2 diabetes mellitus and cardiac surgery

The significance of T2DM in the context of cardiac surgery is that these patients have been demonstrated to have higher rates of postoperative morbidity and mortality (33,34). This is reflected in both the European System for Cardiac Operative Risk Evaluation (EuroSCORE) and Society of Thoracic Surgeons (STS) risk stratification scoring systems (Figure 6a and 6b, respectively), both of which are risk models used to calculate a percentage of mortality in patients undergoing cardiac surgery using seventeen data points pertaining to patient demographics; i.e. their age, gender, creatinine clearance (ml/min), extracardiac arteriopathy, mobility status, previous cardiac surgery, chronic lung disease, active endocarditis, critical preoperative state (defined as requiring 2 or more forms of organ support therapy, and diabetes), baseline condition of the heart (breathlessness and chest pain), left ventricular ejection fraction, recent myocardial infarction, pulmonary artery systolic pressure, operative urgency, surgery involving the thoracic aorta and nature of the cardiac operation (35).

a.



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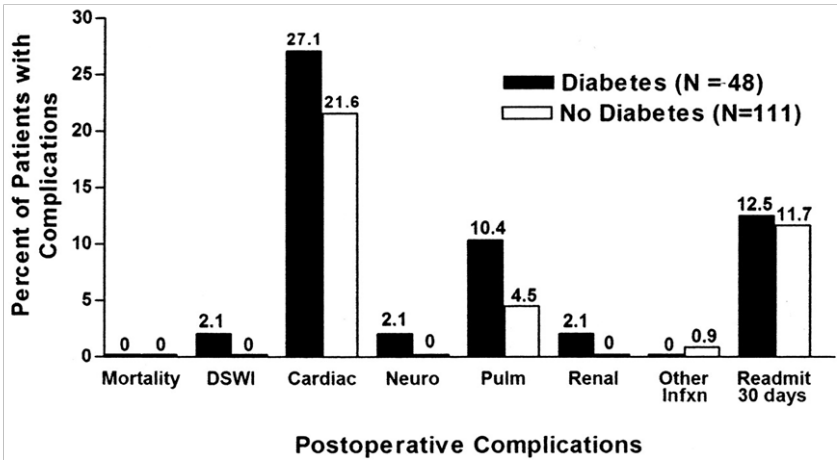


Figure 6. (**a**) Outcomes of complication rates in a cohort (n=180) of postoperative cardiac surgery patients. Mean EuroSCORE II of patients with and without diabetes were 1.2(0.77-1.50) and 0.84(0.6-1.17) respectively. Image credit; Moursi et al. (35). (**b**) Post-cardiac surgery outcomes of a cohort (n=159) of patients scored using the Society of Thoracic surgeons criteria. Mean mortality predictions of patients with and without diabetes were 1.3% (95% CI 0-3.75%) and 2.1% (95% CI 0-8.24%). Image credit; Schmeltz et al. (36)

1.6 The Metabolic Syndrome

The Metabolic Syndrome (MetS) refers to a constellation of interconnected physiological, biochemical, clinical, and metabolic factors that directly increases the risk of atherosclerotic cardiovascular disease, T2DM, and all-cause mortality (101). First described in the 1920s by the Swedish physician Kylin as the triad of hypertension, hyperglycaemia and gout, it gradually evolved to include hypercholesterolaemia and the obesity phenotype (102). The utility of the term MetS was initially confined to little more than an archetypal description of patients seen to be at risk of cardiovascular events and T2DM, as it was widely acknowledged that it was the constituent components of the syndrome, and not the syndrome itself, that was worthy of the clinician's attention and interventional efforts as there was no consensus as to the cause of all these traits manifesting in certain patients (103, 104). Epidemiological studies from around the world highlighted not only the increasing prevalence of MetS transcending ethnicity and socio-economic strata but also the need for formalised criteria in identifying these at-risk patients and establishing therapeutic strategies (105, 107-110). This culminated in the first World Health Organisation (WHO) universal definition of MetS in 1998, citing diabetes, impaired fasting glucose, impaired glucose tolerance or impaired insulin (the latter tested by means of a hyperinsulinaemic, euglycaemic clamp test) with the addition of two or more of the following: (1) Obesity (defined either by a body mass index (BMI) above 30 kg m^{-2} or waist-to-hip ratio greater than 0.9 (males) or 0.85 (females) (2) Dyslipidaemia either by triglycerides levels greater than 1·7 mmol/L, or HDL cholesterol >0·9mmol/L in males or >1·0mmol/L in females (3) Hypertension with a blood pressure of above 140/90mmHg (4) Microalbuminuria with a urinary albumin excretion more than 20 µg/ml. In 1999, the European Group for the Study of Insulin Resistance published similar criteria with the notable omission of BMI in favour of central obesity (waist circumference greater than 94cm in men and 80cm in women. In 2001, the National Cholesterol Education Program's Adult Treatment Panel III (NCEP: ATP III) introduced their criteria for metabolic syndrome without glucose intolerance or insulin resistance as an essential component, with the rationale that progression to T2DM in the presence of these risk

factors alone (without glucose or insulin resistance parameters) was significantly high, warranting early intervention.

These criteria comprised of the following:

1. Blood pressure ≥ 130/85 mmHg or receiving drug therapy for hypertension

2. Fasting serum glucose ≥ 5.6 mmol/L

3. Serum triglyceride ≥ 1.695 mmol/L

4. Serum HDL < 1.04 mmol/L in men and < 1.30 mmol/L in women.

5. Waist circumference of ≥ 102 cm in men and ≥ 88 cm in women).

The NCEP: ATP III criteria is widely used in current clinical practice in the UK today (106). A summary of these criteria is given in Table 2.

Table 2. Different Criteria used in describing metabolic syndrome. The National Cholesterol Education Program's Adult Treatment Panel III (NCEP: ATP III) is most commonly used in cardiovascular clinical practice.

World Health Organisation Universal definition (WHO), 1999	Diabetes or impaired fasting glycaemia or impaired glucose tolerance or insulin resistance (hyperinsulinaemic, euglycaemic clamp-glucose uptake in lowest 25%) *Plus ≥ 2 of the following* i) Obesity: BMI >30 or waist-to-hip ratio >0·9 (male) or >0·85 (female) ii) Dyslipidaemia: triglycerides >1·7 mmol/L or HDL cholesterol >0·9 (male) or >1·0 (female) mmol/L iii) Hypertension: blood pressure >140/90 mmHg iv) Microalbuminuria: albumin excretion >20 µg/ml
European Group for the Study of Insulin Resistance, 1999	Insulin resistance - hyperinsulinaemia: top 25% of fasting insulin values from non-diabetic population *Plus ≥ 2 of the following* i) Central obesity: waist circumference >94 cm (male) or >80 cm (female) ii) Dyslipidaemia: triglycerides >2·0 mmol/L or HDL cholesterol <1·0 mmol/L iii) Hypertension: blood pressure >140/90 mm Hg and/or medication iv) Fasting plasma glucose ≥6.1 mmol/L
NCEP: ATP III, 2001	*≥ 3 of the following* i) Central obesity: waist circumference >102 cm (male), >88 cm (female) ii) Hypertriglyceridaemia: triglycerides >1·7 mmol/L iii) Low HDL cholesterol: <1·0 mmol/L (male), <1·3 mmol/L (female) iv) Hypertension: blood pressure >135/85 mm Hg or medication v) Fasting plasma glucose ≥6.1 mmol/L

The prevalence of MetS is approximately 20-25% in the general population. They are twice as likely to die from coronary artery disease and three times more likely to suffer a heart attack or stroke compared to people without MetS. The exact role of MetS in AS in the absence of coronary artery disease is still under debate.

1.7 Type 2 Diabetes and Aortic Valve Calcification

The similarities of certain molecular processes that are responsible for the shared aetiology between atherosclerosis and calcific aortic valve disease provide a starting point in assessing the influence of T2DM and AS (37). Processes such as non-enzymatic glycosylation of proteins, lipid infiltration (and subsequent oxidization) and activation of protein kinase C, all of which lead to valve leaflet thickening in AS and sclerotic changes in arteries, are the direct consequences of hyperglycaemia (2,38). Differences arise in the structure and arrangement of tissues in the aortic valve, LV outflow tract and aortic root when compared to the walls of arteries and this in turn result in different mechanical and cellular responses to changes in blood volume, flow patterns and pressure (11).

The evidence for Type 2 Diabetes and Aortic Stenosis

Several large studies have demonstrated the significant prevalence of T2DM in patients with isolated AS. Taniguchi and colleagues found that the prevalence of diabetes was 11.4% in a cohort of 3815 patients with severe AS (39). The PRIMID aortic stenosis study found diabetes to be prevalent in 14.4% of the cohort of 176 patients (40). In a study of Medicare beneficiaries in the United States undergoing AVR or TAVI between 2009 and 2015, Culler and colleagues reported an increase in the prevalence of patients with T2DM from 19.7% to 31.6% (41).

Large cohort studies have demonstrated an increased prevalence of T2DM in patients with AS compared to the general population. One of the earliest published manuscripts examining the link

between AS and diabetes was a retrospective study done by Deutscher et al. in 1984 which demonstrated a positive correlation between incidences of T2DM and hypercholesterolaemia in patients with isolated AS (i.e. AS without concomitant coronary artery disease) confirmed on cardiac catheterization (42). This study however did not distinguish between the severity of AS and serum markers of diabetes, neither did it distinguish between T1DM and T2DM. A sample size of fifty-four patients also compromised the powering of this particular study. Nevertheless, this paper remains an important reference as one of the earliest suggestions of the link between T2DM and AS. Aronow et al. performed a larger retrospective study comparing echocardiographic findings of the progression of AS in patients with initial mild AS (i.e. peak systolic transvalvular pressure gradients of between 10 and 25mmHg) (43). All patients had a second echocardiogram two years following the first. A stepwise multiple linear regression analysis of 180 patients demonstrated a positive correlation between T2DM, increasing age, male gender, smoking status, systemic hypertension, serum HDL ≤ mg/dL, serum LDL levels ≥ 125mg/dL and concomitant statin use. Limitations of this study pertained to uneven gender distribution as it involved 125 women and 55 men, and data on pre-existing coronary artery disease in these patients was not declared.

Large observational studies in the last few years by Larsson et al. (44) and data from the 2017 CANHEART aortic stenosis study provided more robust evidence of not only the association of T2DM and AS, but also established a positive dose-response relationship between the severity and chronicity of T2DM and severity of AS. The CANHEART study excluded patients with pre-existing coronary artery disease, heart failure arrhythmias, cerebrovascular disease and other valve pathologies. This study identified hypertension, diabetes, and dyslipidaemia as independent, dose-dependent factors in significantly increasing the risk of developing severe AS in 20,995 patients. The combination of these risk factors accounted for approximately one-third of the attributable risk for AS in the population studied. One major limitation of this study was that the data points of severe AS were transcribed from hospital admission coding, rather than from echocardiographic findings, resulting in only a binary assessment of AS status. This prevented analysis of peak and mean

transvalvular gradients, left ventricular (LV) outflow tract velocities, aortic valve area (AVA) and LV mass indices. Such data, when combined with chronicity of the aforementioned risk factors would have added valuable insight into the ascertaining influence of T2DM (and the other risk factors of cardiovascular disease) on the rate of AS progression. A large prospective cohort study conducted by Larsson et al. (45) of 71,483 participants from the Cohort of Swedish Men and Swedish Mammography cohort databases endeavoured to establish the relationship between T1DM and T2DM and incidences of the unfavourable cardiovascular outcomes of AS, atrial fibrillation, abdominal aortic aneurysm, and intracerebral haemorrhage while adjusting for potential confounding factors such as adiposity, smoking, diet, levels of physical activity on alcohol consumption alongside other conventional biochemical cardiovascular risk factors. This study found that T2DM but not T1DM was associated with an increased risk of AS, although it must be emphasized that this may be attributed to the small number of patients with T1DM and AS in this cohort study (n=4). Body mass index (BMI) was cited as an important confounder in this study, suggesting a link to the metabolic syndrome as a whole, rather than the isolated incidence of T2DM. This is in contrast to the independent relationship of both T1DM and T2DM with myocardial infarction and ischaemic stroke. Interestingly, atrial fibrillation was only associated with long-term (≥20 years) prevalence of T2DM. This may be accounted for by gradual but progressive distortion of left ventricular geometry in diabetic cardiomyopathy which differs in morphology from that of T1DM. Overall, this study is invaluable as it is one of the biggest datasets investigating the link between T2DM and AS. A prospective cohort study involving 5079 subjects by Martinsson and colleagues confirmed low-density lipoprotein cholesterol, smoking, presence of carotid intimal plaque, C-reactive protein, BMI and diabetes mellitus as significant predictors of incident AS in 69 subjects at 20 years by age and sex-adjusted multivariate analysis (6). A potential source of underrepresentation of the true prevalence of AS in this study is due to AS data being collated from a registry database of patients who have been referred for investigation for symptomatic AS, and not from baseline echocardiography for all participants. Also, as no data on glycaemic status (i.e.

HbA1c levels) and chronicity (i.e. duration of diabetes) was recorded, no association between disease severity and AS can be ascertained from this otherwise invaluable population cohort.

An important longitudinal study by Kamalesh and colleagues demonstrated a correlation between in progression of the severity of AS in patients with and without diabetes (46). In this imaging study, the authors utilised serial M-mode (Figures 7a & 7b), continuous- and Doppler-wave (Figure 7c) echocardiography techniques to tabulate the progression of AS severity using a variety of geometric and haemodynamic parameters in 166 patients.

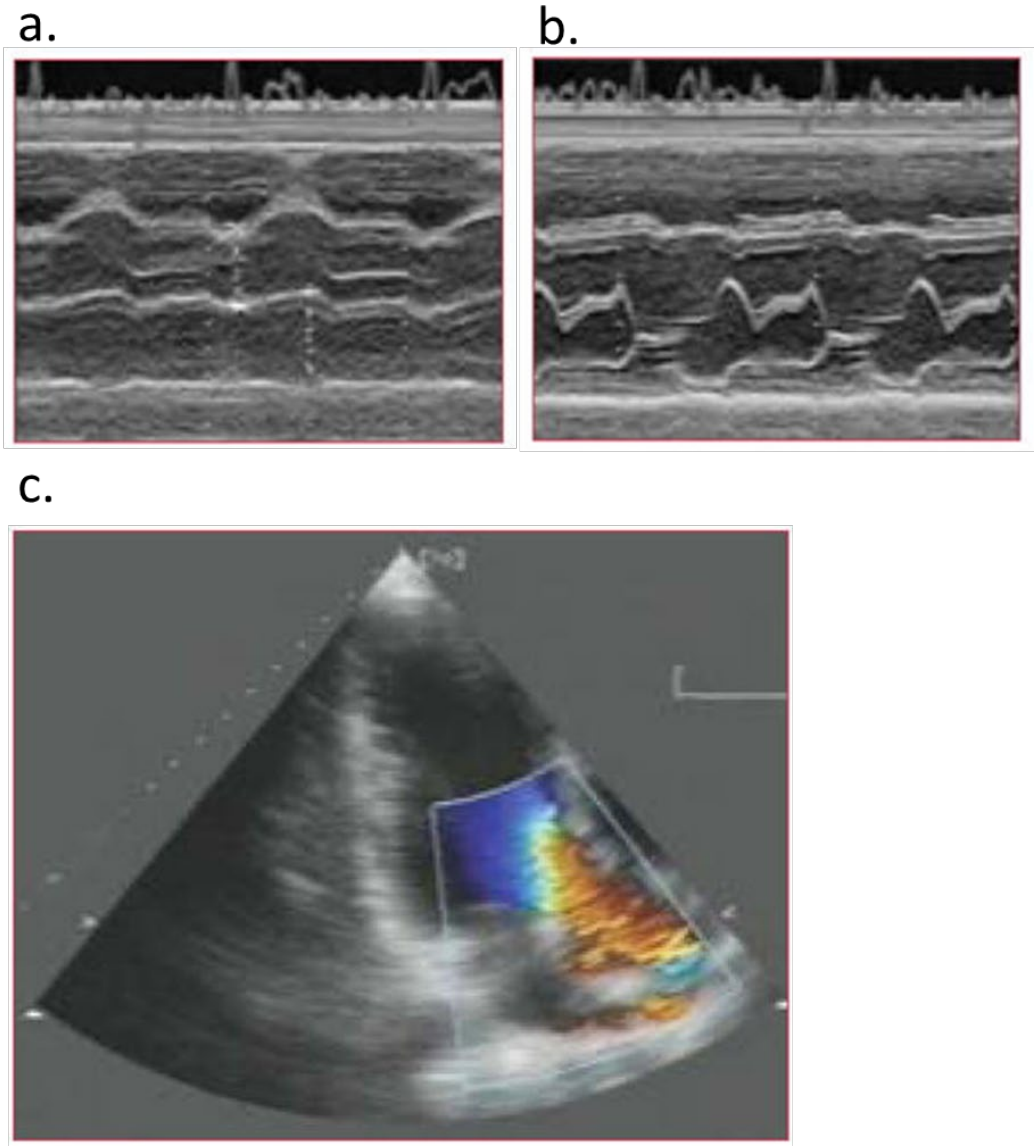


Figure 7. Example of motion mode (M-mode) image of (**a**) the aorta/left atrium and (**b**) the mitral valve, both in a healthy heart. M-mode provides a one-dimensional view with high temporal and spatial resolution, facilitating better accuracy for measurement purposes. (**c**) Colour-flow Doppler allows for the superposition of colour onto 2-dimensional echocardiographic images to assess fluid flow velocities. Image credit; Ashley and Niebauer (47)

As all participants have baseline echocardiography recordings, comparisons of AS progression between patients with and without T2DM could be calibrated with individual baselines of peak and mean aortic valve gradients and aortic valve area (AVA). The results revealed an interesting bell-shaped correlation between AS progression in the two groups. There was no difference in the rate of progression in patients with baseline mild or severe AS, however diabetic patients with moderate AS at baseline demonstrated significantly accelerated progression compared to their non-diabetic counterparts, based on the reduction in AVA. It is noteworthy that deterioration in peak and mean aortic valve gradients was more pronounced in diabetic patients, although statistical significance was not achieved in this study. It is recognised, however, that AVA is the more reliable parameter that correlates to the burden of valve stenosis in contrast to measurements of valve gradients as the echocardiographic assessment of the former is not compromised by incidental fluid dynamics anomalies such as decreased stroke volume, contractility issues and anaemic states as it is in the latter. As calcification of the aortic valve is a multi-mechanistic process, these findings lend credence to the hypothesis that diabetes may play a role in driving molecular processes in the middle or secondary phase of calcification (involving lipid infiltration, inflammation and oxidation – discussed in the next section) rather than instigating early valvular changes (i.e. via endothelial injury or activation of valvular interstitial cells).

A large prospective cohort study yielded contrasting results to the others mentioned above (48). The Tromso Study – eponymously named after the region where it was conducted - involved randomly-selected members of the population of a Norwegian municipality who underwent three echocardiographic assessments over a period of 14 years, and identified age, systolic blood pressure (BP), smoking status, and waist circumference as independent predictors of incident AS. No statistically significant correlation between diabetes or hyperglycaemia with the incidence of AS was demonstrated in this cohort, however, these results are of limited value as only six patients with diabetes in this cohort of 3243 participants developed AS. Extrapolation of these results to any other population must be done with caution, as data was collected from the sole cardiovascular institute

in the said municipality, encompassing a geographical area of 2521km^2 and a population of 75638 (48). Nevertheless, these results may allude to genetic and environmental influences on the incidence and progression of AS.

Another prospective cohort study found no correlation between T2DM and metabolic syndrome (MetS) with the progression of AS (49)**.** This study enrolled 203 patients with varying grades of AS irrespective of symptomatic status with a minimum of a 2-year follow-up period, and valve surveillance was performed using both transthoracic echocardiography (TTE) and multi-slice computed tomography (CT) annually. Baseline demographics including cardiovascular risk factors and metabolic syndrome were tabulated, and outcomes of death, development of congestive heart failure as well as the onset of new AS symptoms (i.e. angina, dyspnoea and syncope) were prospectively recorded. Using a combination of the echocardiographic parameter mean pressure gradient across the valve (MPG) and Agatston method (AU) for calculating valvular calcium scores by CT scan. Briefly, this is a semi-automated tool utilizing unenhanced low-dose computed tomographic images to calculate calcification density by multiplying the highest attenuation value and area of calcification speck (50). The results demonstrated that AS hemodynamic progression by TTE was not different between patients with MetS. Neither was there a difference in progression by assessment of valve calcification by CT scan. Similar results were observed when assessing the influence of T2DM on AS progression, with no significant mean MPG progression on TTE and no significant change in valve calcium score. Subgroup analyses based on age, statin prescription, and valve anatomy or AS baseline severity also failed to show any association in patients with T2DM, MetS and a combination of the two. The contrasting conclusion of this study can be attributed to differences in study design, as only patients with pre-existing aortic valve stenosis were recruited, whereas the larger longer-term observational studies quote above have the advantage of detecting *de novo* AS in previously healthy patients. Despite the relatively small sample size (n=203) in this study, it is reasonable to hypothesize that T2DM and/or MetS may play a more active role in the initial stages of aortic valve calcification. This may account for the findings of increased risk of AS in other studies; nonetheless

the finding of no significant change in the rate of progression in this cohort remains at odds with the aforementioned studies.

Despite the balance of evidence being in favour of T2DM being associated with increased incidence and rate of progression of AS, it is by no means conclusive. This in part is due to statistical models being unable to accurately account for the weighting of the other cardiovascular risk factors on the magnitude of metabolic and biochemical underpinnings of the final disease state. Epigenetic variation in metabolic processes presents another hurdle, as standardization of geodemographic factors such as ethnicity, environment, diet and lifestyle factors makes extrapolation of results from one cohort to a universal generalisation impossible. It is unlikely that large randomised-controlled trials assessing treated and untreated T2DM on a cohort of geodemographically-identical patients with isolated AS and no other risk factors will ever be carried out due to practical (and more importantly, ethical) considerations. Therefore, population-based observational and longitudinal studies assessing the strength of the association of individual elements with specific outcomes are still warranted to further develop an understanding of the pathological interplays of T2DM and AS.

1.8 Left Ventricular Remodelling in Type 2 Diabetes and Aortic Stenosis

The important common pathological sequelae in T2DM and AS is the phenomenon of left ventricular remodelling; a term used to denote dimensional and structural changes of the LV which is initially compensatory, but in time ultimately deleterious to the function of the heart in matching cardiac output to physiological demands of various organ systems (51). The stiff, narrowed aortic valve in AS provides resistance to ejection of blood from the LV chamber (a prerequisite to this is a competent mitral valve, preventing regurgitation of this blood into the left atrium – this is the norm in the early stages of the disease). Over time, the chronic increase in pressure against the chamber walls provides the stimulus responsible for compensatory hypertrophy of the myocardium, enabling stronger recoil during ventricular systole thus overcoming the resistance caused by outflow

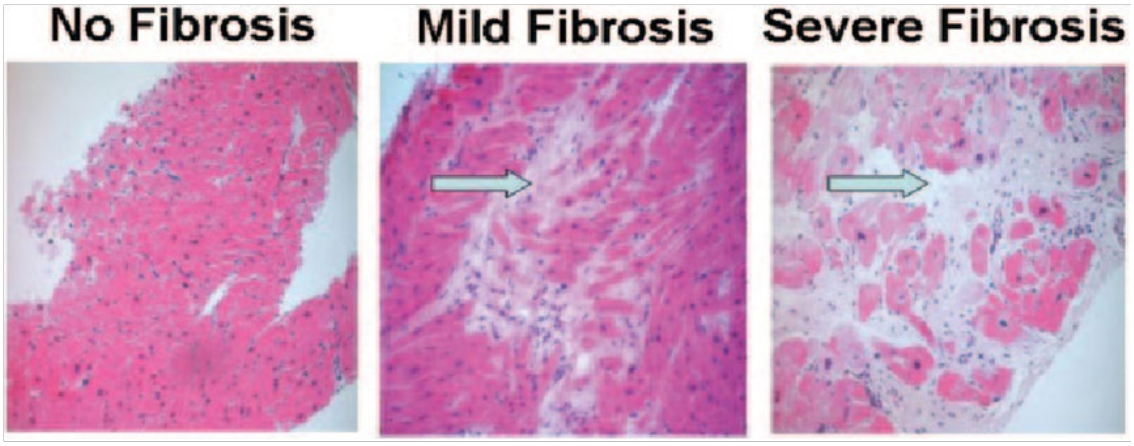
obstruction. It has been suggested that chronically increased pressure in the ventricle triggers re-expression of the dormant TEAD-1 gene (which was involved in the initial growth and differentiation of the myocardium) which result in an increase in cell volume (52). When the ratio of ventricular wall thickness to chamber size is increased, this is termed concentric hypertrophy. This remodelling is also seen in hypertension and coronary artery disease where - despite the absence of mechanical resistance to outflow from a stenotic aortic valve - high ventricular pressures are generated as a result of ejection impedance due to increased systemic vascular resistance (SVR) in the case of the former, and impaired strength of contractility of ventricular myocardium due to suboptimal oxygen delivery due to impairment of coronary arterial flow in the latter (53).

1.9 The Diabetes Paradox - Left Ventricular Remodelling in the absence of Aortic Stenosis

T2DM patients without AS also undergo concentric hypertrophy of the LV, despite the absence of LV outflow tract obstruction (54). This is also independent of the other cardiovascular conditions causing increased LV filling pressures such as hypertension and coronary artery disease as mentioned above, suggesting that mechanisms other than chronic pressure overload may be responsible. There is emerging evidence of excess cytokines proliferation in patients with impaired glucose tolerance, the source of these cytokines being increased adipose tissue(55). Another hypothesis centres on the role of insulin signalling as a potential growth factor in the heart. This is supported by experimental evidence of a reduction in cardiac size following the deletion of insulin receptors (56). The increase in intramural myocardial triglyceride and cholesterol levels may also play a role as patients with T2DM, obesity, insulin resistance and impaired glucose tolerance demonstrate increased intramyocardial lipid content which bears no correlation to levels of circulating triglycerides (57). When LV remodelling occurs as a result of the replacement of cardiomyocytes with fibroblasts and excess deposition of extracellular (ECM) proteins, it is termed myocardial fibrosis (58). The unfavourable sequela of this is deteriorating myocardial contractility,

leading to an increased risk of adverse cardiac events including mortality. Fibrosis of cardiac interstitial and perivascular tissue, alongside an increase in the deposition of collagen and subsequent cross-linking of these fibres, occurs in patients with T2DM (59). These structural changes are evidenced as an increase in the mass of the heart as evidenced by echocardiographic and cardiac magnetic resonance imaging (CMR) studies (60).

a.



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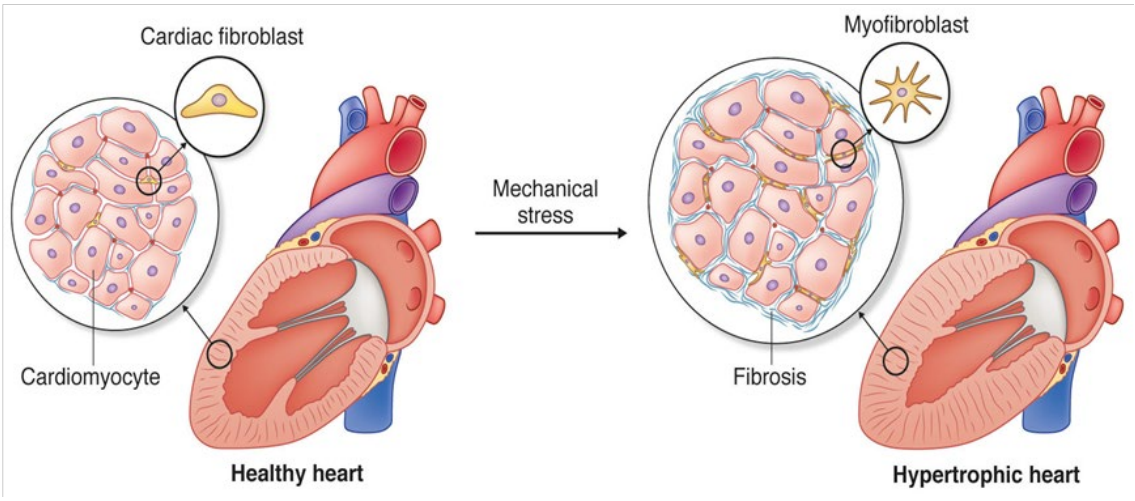


Figure 8. (**a**)Typical histology (hematoxylin and eosin staining) from endomyocardial biopsies showing no, mild, and severe fibrosis. Biopsies were taken intraoperatively through the LV outflow tract from the endocardium of the basal LV septum. Arrows indicate fibrosis. Image and explanation credit; Weidemann et al. (61). (**b**) Cardiac fibroblasts are located in between cardiomyocytes where they ensure the appropriate amount and composition of extracellular matrix (ECM) in the healthy heart. Mechanical stress induces fibrosis during cardiac remodelling, e.g. hypertrophic remodelling. Fibrosis compromises cardiac function and results from the activation of cardiac fibroblasts and transition into a myofibroblast phenotype characterized by excessive production of ECM. Image and explanation credit; Herum et al. (62)

1.10 Evidence for the role of glycaemia and insulin resistance in LV hypertrophy

Velagaleti and colleagues sought to conduct a volumetric assessment of the left ventricle by CMR in 1603 patients (63). Glycaemia, insulin levels and insulin resistance were tested in all subjects and 8 to 12 contiguous 10-mm-thick LV short-axis images were acquired using CMR to obtain measurements for calculation of LV end-diastolic volumes (EDV) and end-systolic volumes (ESV). Indexing these measurements against the patient's height allowed for the calculation of LV mass (LVM). Results showed that in multivariable-adjusted models for age, BMI, systolic blood pressure, smoking status and use of 'cardioactive' drug therapies (defined by the authors as medications which have confirmed evidence of favourable LV remodelling; these were angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, or aldosterone antagonists), both men and women had statistically significant reductions in left ventricular mass index (LVMi), LV ejection fraction (LVEF) and relative LV wall thickness (RWT) with insulin resistance. Age-adjusted modelling demonstrated a significant correlation between left atrial diameter (LAD) and cardiac output (CO) with insulin resistance. Analysis of covariance (ANCOVA) elicited the combination of glycaemic deterioration and increasing insulin resistance as independent influencers of concentric LV hypertrophy, and this was found to be independent of age, gender, systolic blood pressure and BMI. Data on the glycaemic status and insulin resistance was from contemporaneously acquired samples at the time of CMR imaging, providing a snapshot of LV status with disease burden. As there was no serial sample measurement and imaging follow-up, no conclusion can be drawn regarding the influence of glycaemic and insulin resistance status with the progression of LV remodelling. Oral glucose tolerance tests were only performed on one-third of the cohort, therefore limiting analysis of correlation between 2-hour glucose and insulin measurements to CMR findings.

Data from a subgroup analysis of patients from the Multi-ethnic Study of Atherosclerosis (MESA) prospective cohort study mirrored the findings above (64). 5004 cardiac disease-free participants from four different ethnic backgrounds in the United States (white, African American, Hispanic and Asian of Chinese descent) underwent extensive baseline evaluation of cardiac risk factors alongside

CMR assessment for left ventricular mass, volume and systolic function. Assessment of glycaemic status stratified participants into three separate categories (diabetes, impaired glucose tolerance and normoglycaemia). The association of mean measurements of LVM, end diastolic volume, stroke volume, ejection fraction and cardiac output was adjusted for five sociodemographic characteristics (age, gender, race, physical activity and geographic location) and height. Results after adjustment for the aforementioned variables increased significantly LV mass in T2DM, reduced stroke volume and lowered ejection fraction. A similar association was found in patients who were current smokers (increased LV mass), reduced stroke volume and reduced ejection fraction. The significance of this study is the recognition of subclinical LV remodelling and its strength of association with T2DM and other conventional cardiovascular risk factors in four different ethnic populations with no cardiac disease, highlighting the significance of diabetes as a risk factor irrespective of ethnic background. As BMI was used as a surrogate for body size, detailed scrutiny of the effect of adiposity on LV mass was hindered due to the absence of lean body mass and fat mass measurements. Such measurements, particularly in diabetes, would have allowed for the indexation of CMR parameters with not only quantity but also the distribution of body adipose tissue.

Another large study derived from the Framingham Offspring Cohort corroborated these findings (65). This cohort (n=2623) was free from prevalent myocardial infarction, heart failure and renal insufficiency. Measurements of LV geometry parameters were acquired from M-mode two-dimensional echocardiography, performed independently by two experienced sonographers who were blinded to details of participant demographics in an effort to examine the relationship between glucose tolerance and insulin resistance with LV and left atrial (LA) measurements. Glucose tolerance was subcategorized into four quartiles. Covariate-adjusted LV mass correlated with deteriorating glucose tolerance. However, when adjusted for BMI, this relationship was no longer significant in men. In both women and men, LV end diastolic diameter (LVEDD) and LV wall thickness (LVWT) increased across the groups in models without adjustment for BMI, but this relationship was attenuated on adjustment for BMI. Increase in LA size showed a direct correlation with increasing

BMI in both sexes, however when atrial fibrillation (AF) and mitral valve regurgitation (MR) were factored in, this relationship was no longer statistically significant in men. An important implication of these results is the role of obesity, a modifiable risk factor, in changes in LV geometry in women with impaired glucose tolerance and insulin resistance. The choice of echocardiography as an imaging modality may have limited analysis on the impact of BMI or adiposity on LV measurements, as the authors note that the majority of excluded echocardiography results were due to suboptimal image quality in patients with increased BMI (29.6kg/m^2) compared to the mean BMI of recruited participants (26.8kg/m^2).

Not all studies of insulin resistance and LV remodelling have yielded these results. As previously discussed, increased systemic vascular resistance (SVR) is one pathophysiological mechanism of chronic LV pressure overload and although hypertension is assumed to be responsible, it is not the sole aetiology that may cause an increase in SVR. Vascular (arterial) hypertrophy, an early compensatory mechanism of chronic systemic pressure overload, acts as a stimulus via a feedback mechanism that induces LV remodelling in the absence of - or more commonly in the early, latent stages of – systemic hypertension. The AT_1 subtype of angiotensin-II activates the G protein, phospholipase C, diacylglycerol and inositol trisphosphate pathway, increasing expression of the proto-oncogenes and growth factors (platelet-derived growth factor-A-chain, transforming growth factor-beta 1 and basic fibroblast growth factor) and can result in both cellular hypertrophy and hyperplasia (66). Olsen and colleagues examined the triad of insulin resistance, vascular hypertrophy (remodelling of peripheral small and larger conduit arteries) and LV hypertrophy in patients with systemic hypertension (67). Ninety-nine patients with essential hypertension and evidence of LV hypertrophy were confirmed by electrocardiography. Of these, ten patients had diabetes and 22 had been newly diagnosed with essential hypertension. All participants had baseline blood tests, echocardiography, ultrasound imaging of the common carotid arteries, strain gauge plethysmography measurement of minimal forearm vascular resistance (MFVR), and underwent a hyperinsulinaemic clamp challenge. Briefly, this is a method of measuring insulin sensitivity whereby

infusion of a constant concentration of insulin is infused into a large peripheral vein alongside a variable concentration of glucose – in this case 200g/L - until venous blood sampling demonstrates a steady state of glucose uptake by tissues (68). Seventy-eight percent of the cohort demonstrated abnormal LV geometry, and results showed that cardiovascular hypertrophy measured as LV mass index, cross-sectional area of intima-media thickness indexed by height and MFVR in male patients, were all positively related to systolic blood pressure. Measurements of LVEDD, LVM and LV mass index (LVMi) correlated with common carotid artery hypertrophy but not with MFVR. In terms of glycaemic status, LVMi correlated negatively to serum glucose and serum insulin and demonstrated a tendency towards a positive (but statistically insignificant) correlation to the whole-body glucose uptake index. These findings of no correlation of both systolic and diastolic LV function with glucose levels, insulin levels and insulin resistance is contradictory to other studies. The authors have hypothesized that this is likely to be a result of a cohort with established hypertension and LV hypertrophy, as metabolic influences of remodelling are predominantly responsible for early remodelling changes.

Devereux and colleagues sought to investigate the relationship between insulin and LV hypertrophy in patients with hypertension, but without T1DM or T2DM (69). Baseline demographic measurements, data on lipid profile, fasting glucose and insulin levels and blood pressure. Two-dimensional phased-array echocardiography (M-mode, pulsed continuous wave and colour Doppler) was the investigation of choice, and parameters of interest were LVEDD, RWT, LAD, aortic root diameter (AoD) and valvular regurgitation (mitral and aortic). Measurements were scrutinised by two (one experienced) sonographers blinded to patient demographic details. Statistical analyses involved the use of Pearson's and Spearman's correlation between log insulin and clinical parameters with LV measurements, and regression analyses for age, gender, BMI, blood pressure, height, and different classes of hypertensive medications. Results from this cohort of 1542 participants demonstrated a weak but positive correlation of plasma insulin levels with posterior LV wall thickness (PWT), but no correlation with interventricular septal (IVS) thickness. The same weak

positive correlation was seen between insulin levels and LV mass (LMV), LV Mass indexed for height LVMi, and relative LV wall thickness. With regards to demographics, log insulin levels showed a statistically-significant correlation to body mass index, more so in men than women. This correlation was even stronger in Caucasian populations compared to African American populations. LV mass was strongly correlated with BMI, and once again this correlation was more pronounced in men compared to women. Conversely, LV mass was significantly increased in African Americans compared to Caucasian individuals. The weak associations of plasma insulin levels with parameters of LV remodelling in this cohort of hypertensive, non-diabetic patients hints towards the significance of established diabetes and LV remodelling, despite the knowledge that hypertension alone may be sufficient to instigate changes of concentric hypertrophy in the ventricle, no statistically significant correlation between hypertension and LV remodelling was demonstrated.

Focused studies examining the link between T2DM and AS are limited, and this is likely due to a difficulty in establishing whether remodelling activity is truly a result of chronic pressure overload in the LV (resulting from the LV outflow tract obstruction of AS) or mechanisms of true diabetic cardiomyopathy, and it is possible that both processes may act synergistically in causing accelerated ventricular hypertrophy. Nevertheless, efforts to distinguish between the two remain important not only for prognostication but also for guiding potential future targeted therapy of one or both major pathways.

1.11 Cellular mechanisms in Aortic Stenosis

The futility of lipid-lowering therapies in decelerating disease progression in moderate to severe AS by four randomised-controlled trials highlighted the limitations of parallel modelling of this disease with atherosclerosis, and hence generated renewed interest in the exploration of molecular and cellular processes underpinning AS (70-73). Isolated experimental studies have successfully uncovered several distinct mechanisms responsible for various components ultimately leading to calcification (see Figure 9), however definitive modelling of these processes into a validated "cascade" of valve calcification remains elusive, due to the inability of ascertaining the precise chronology of events (74).

A simple template separating major cellular changes in the instigation and propagation of AS pathological processes serves as a useful starting point. Briefly, endothelial injury to the outermost monolayer of aortic valve leaflets - either due to the effects of haemodynamic shear stress or dysfunction of valvular endothelial cells – attracts the accumulation of inflammatory cells and lipids(75). Lipid deposition (and subsequent oxidation) in turn propagates further infiltration of inflammatory products, thus giving rise to a localised chronic inflammatory milieu. This then results in the activation of various cytokines (transforming growth factor $\beta 1$ and Interleukin -1β) which causes a localised rise in the matrix metalloproteinases (MMPI and MMPII), culminating in cell apoptosis (23).

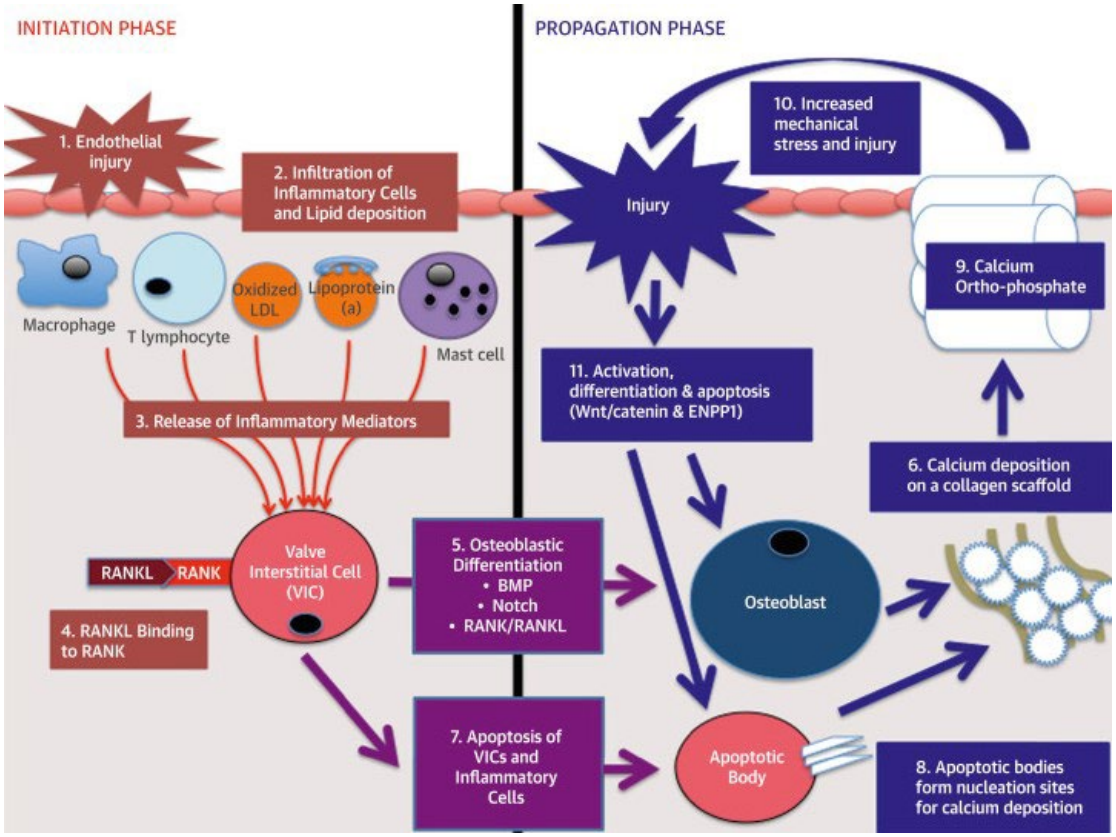


Figure 9. Cellular mechanisms in the initiation and propagation phase of calcification. BMP, bone morphogenetic protein; ENPP1, ectonucleotide pyrophosphate 1; LDL, low-density lipoprotein; RANK, receptor activator of nuclear kappa B; RANKL, receptor activator of nuclear kappa B ligand; RAS, renin-angiotensin system; VIC, valvular interstitial cell. Image credit; Pawade et al. (15)

Separately, an increase in angiotensin-II concentrations (either from the angiotensin-converting enzyme, chymase, cathepsin G or mast cells – is triggered alongside increased expression of the Thioredoxin interacting protein gene (TXNIP) (Figure 10). Uncoupling of nitric oxide also occurs causing the generation of reactive oxygen species. These mechanisms are responsible for two processes which ultimately lead to valvular fibrosis; an increase in oxidative stress and the replacement of apoptotic cells with fibroblasts (which then differentiate into myofibroblasts) (14). Ossification of the valve then ensues as secretion from extracellular matrix proteins promotes the activation of osteoblasts and production of osteopontin, osteocalcin, osteonectin and bone

morphogenic protein 2 (BMP-2) (76). These extracellular matrix protein secretions also result in the inhibition of the important anti-calcific proteins fetuin-A and matrix Gla-protein (MGP).

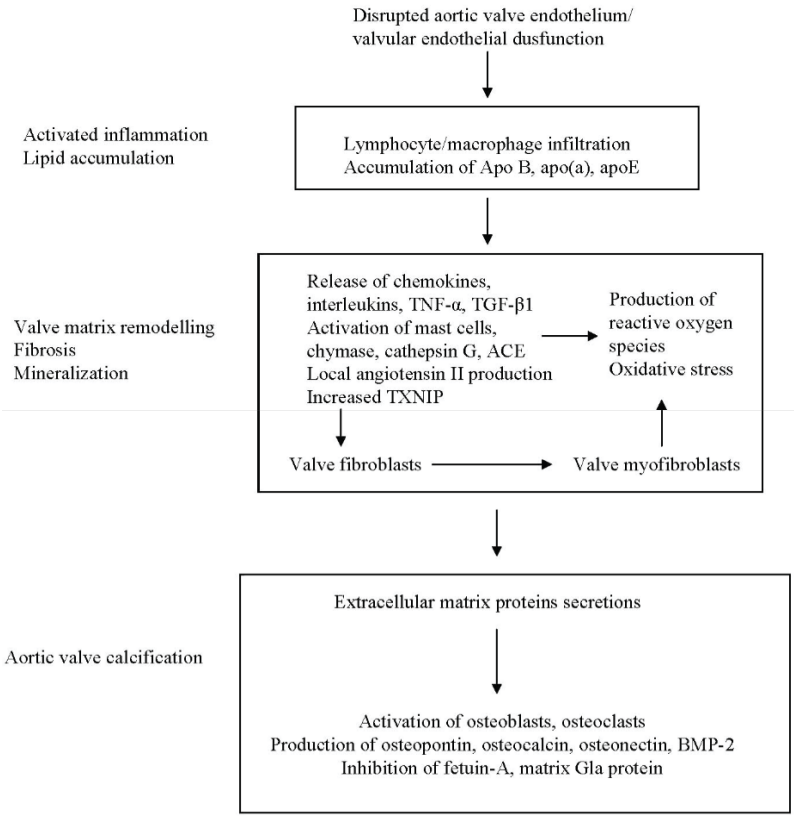


Figure 10. Postulated mechanisms underlying aortic valve lesion formation. Inflammatory infiltrations of T-lymphocytes and macrophages, along with lipid accumulation, are responsible for the early thickening of aortic valves. Interactions between chemical stimuli and disruption of valvular homeostasis: pro- and anti-fibrotic mechanisms. Later stages of aortic stenosis: - cytokine release and angiotensin II promote extracellular matrix proteins secretion at early stages of mineralization which in turn begin the processes of bone formation. This process occurs largely at the end stage of aortic stenosis where aortic valve mobility is significantly reduced due to a build-up of bone-like calcific nodules. From Sverdlov et al. (23)

1.12 The lipid theory – A unifying theme?

Table 3. Studies assessing components of the lipid pathway in relation to aortic stenosis.

Study	Study	Results
CHR study	1991	Cholesterol and related lipoproteins are independent risk factors of AS (77)
Parhami et al.	1997	In vitro, oxidized lipid products and hypercholesterolemia has induced aortic valve calcification and stenosis
Kamath et al.	2008	AS progresses twice as rapidly in cases of hypercholesterolemia (78)
MONICA/KORA study	2009	Hypercholesterolemia and active smoking were significantly related to AS at follow-up (79)
Capoulade et al.	2012	MetS induces more rapid progression of AS than for those without MetS(80)
Weiss et al.	2013	RANK/RANKL/Osteoprotegerin induce myofibroblasts modification to osteogenic components (81)
Epic-Norfolk, CCHS, CGPS studies	2014	Elevated Lp(a) levels increase the risk of aortic valve calcinosis(82) (83)
CHARGE study	2014	Lp(a) directly induces calcinosis of aortic valve and progression of AS. Genetic risk score of LDL was significantly associated with calcinosis of aortic valve (84)
Vongpromek et al.	2015	Hypercholesterolemia induced aortic valve calcinosis and AS (85)
Parisi et al.	2015	Increased LDL levels activate calcinosis of aortic valve (86)
MESA study	2016	Lp(a) levels are associated with aortic valve calcification (87)
Rajamannan et al.	2016	Lp(a) levels are associated with DAS by genetic variations (88)
Thanassoulis et al.	2016	Targeted Lp(a) therapy may become a new opportunity to treat AS (89)
Larsson et al.	2017	Obesity is associated with increased risk of AS (90)
Larsson et al.	2017	Risk of DAS increased with increasing smoking intensity and former smokers had similar risk for AS as non-smoker (91)

Histochemical analyses have confirmed the abundance of oxidised low-density lipoproteins (LDL) and lipoprotein-A, Lp(a) in excised human calcified aortic valves, and given the epidemiological correlation between elevated serum LDL levels in both patients with T2DM and AS, this pathway may account for one of the common mechanisms in these two disease processes (86). Evidence of

diffuse atherosclerotic lesions in both aortic valve leaflets and coronary arteries in patients with familial hypercholesterolaemia attests to the key role of lipids in vascular calcification (92). This retention of lipids in turn is hypothesized to be a result of proteoglycan excess, as biglycan and decorin have been shown to be overexpressed in calcified aortic valves (93). Biglycan, in particular, has been shown to demonstrate overexpression in patients with concomitant AS and T2DM. Elongation of these proteoglycan chains allows prolonged interaction with lipoproteins, namely apolipoprotein-B, apolipoprotein-E and apolipoprotein-A1, facilitated by tumour necrosis factor TNF-β.

In T2DM, increased levels of circulating lipids are the product of hepatic steatosis – an increase in the content of hepatocellular lipids – and insulin resistance (94). It has been hypothesized that hepatic steatosis is responsible for insulin resistance due to a combination of the diversion of excess fatty acids to the liver and decreased levels of adiponectin (a protein hormone synthesized by adipose tissue which is involved in the regulation of serum glucose and the breakdown of fatty acids). These in turn result in the activation of pro-inflammatory pathways (i.e. protein-C kinase), transcription factor nuclear factor κB, and c-Jun N-terminal kinase-1. Adiponectin displays both anti-atherogenic and anti-diabetic properties, as it promoted the release of NO by vascular endothelium, modulated macrophage function and inhibits the production of TNF-α and IL-6 (76). There is evidence of the protective effect of adiponectin in animal models studying vascular calcification, however, initial research on the effect of adiponectin in inhibiting myocardial remodelling is conflicting (69,95). Conversely, leptin and resistin are both proinflammatory and proatherogenic hormones (96). Leptin stimulates smooth muscle proliferation, induces oxidative stress, promotes endothelial dysfunction, and is implicated in LV remodelling (97). Paradoxically, leptin has demonstrated an association with decreased cardiac lipid accumulation (98). Resistin is responsible for increased levels of circulating LDL and insulin resistance and has been shown to impair the action of HMGCoA reductase inhibitors (statins), hinting at a possible explanation of the inefficacy of statins in patients with AS.

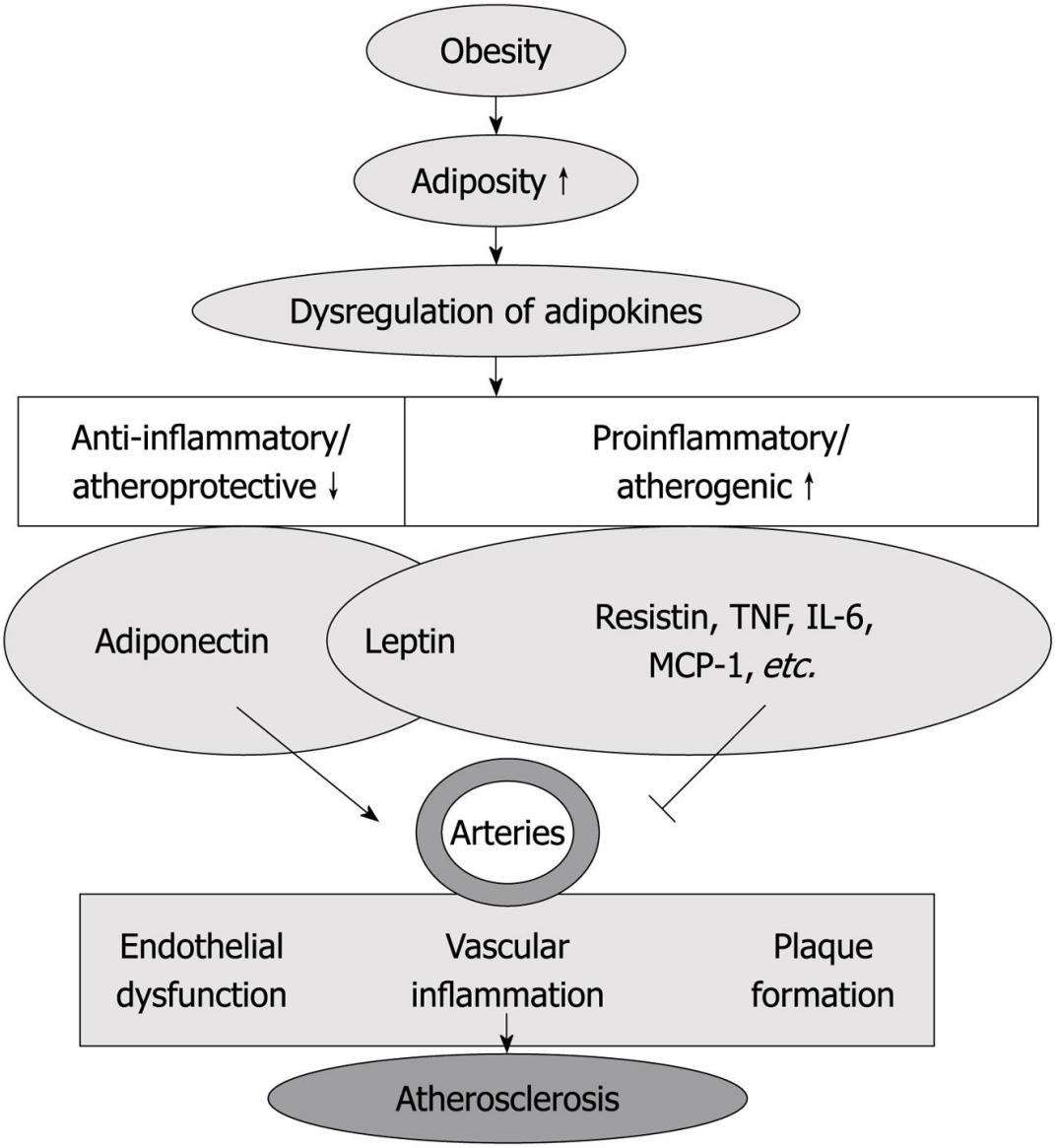


Figure 11. Increased adiposity (obesity) is associated with dysregulated adipokine production, which is characterized by a decrease in anti-inflammatory/atheroprotective adipokines (adiponectin) and an increase in proinflammatory/atherogenic adipokines [resistin, tumor necrosis factor (TNF)-α, interleukin (IL)-6, macrophage chemoattractant protein (MCP)-1, etc.]. Those adipokines participate in the regulation of endothelial function, vascular inflammation and plaque formation, which contribute to the inception and progression of atherosclerosis. The exact pathway mechanism of adipokine activity in relation to isolate AS has not been established. Image credit; Zhang et al. (99)

The subfamily of small leucine-rich proteoglycans (SLRP) and their role in lipid retention are relatively novel and warrant further scrutiny. Biglycan plays a regulatory role in the assembly of extracellular matrix molecules such as elastin and collagen resulting in fibrosis, heralding a potential link between insulin resistance and LV hypertrophy. Barth and colleagues sought to elucidate the relationship of SLRP upregulation in calcified human aortic valves and glycaemic status using reverse-transcription polymerase chain reaction, Western blot, enzyme-linked immunosorbent assay, immunohistology and colorimetric assays. Results demonstrated that only biglycan was upregulated in calcified valve specimens alongside osteocalcin and alkaline phosphatase, and these expressions were significantly higher in patients with T2DM.

The lipid theory may also provide some degree of explanation of LV remodelling in patients with AS and T2DM. Leptin has been demonstrated to cause cardiac hypertrophy in vitro. The postulated mechanism for this is endothelin-1 reactive oxygen species (ROS) generation. The same remodelling has been demonstrated with resistin, though it is hypothesized that insulin receptor substrate-1 (IRS-1) and mitogen-activated protein kinase (MAPK) signalling pathways are implicated. Another lipid-associated pathogenic factor is myocardial lipotoxicity, which leads to cellular apoptosis and replacement fibrosis. Deposits of lipofuscin have been seen in transmural biopsies of interventricular septums of diabetic hearts, demonstrating significantly elevated levels of myocardial triglyceride (TG) and cholesterol content. The association of these elevated lipids in T2DM, obesity and insulin resistance was independent of circulating triglyceride levels, suggesting a localised process. In mouse models, overexpression of proteins involved in cardiac fatty acid transport such as long-chain Acetyl-Coenzyme-A synthetase, glycosyl-phosphatidyl-inositol (GPI) membrane-anchored lipoprotein lipase or fatty acid transport protein 1 resulted in lipotoxic cardiomyopathy. Interstitial fibrosis, a hallmark of diabetic cardiomyopathy, is characterised by an increase in Type III collagen and pro-collagen Type I carboxy-terminal peptide, and Zuo and colleagues have demonstrated increased expression of TGFβ1 receptor density (100). As mentioned previously, increased lipid deposition and oxidation cause activation of TGFβ1, thus linking this mechanism to the lipid model.

It must be emphasized that various other cellular and molecular mechanisms are simultaneously involved in the complex interplay of pathways that lead to aortic valve calcification and myocardial fibrosis in patients with T2DM, though these pathways are distinct for either calcification or fibrosis. As patients with AS and T2DM often present a constellation of other metabolic stressors such as increasing age, hypertension, coronary artery disease, obesity and smoking, it is plausible that the earlier onset and accelerated progression witnessed may be merely a cumulative result of the separate bio-molecular processes resulting from these independent aetiologies. Although the lipid theory is able to account for virtually all currently known molecular pathways in these patients, a more focused and thorough evaluation is needed. Unlike pathways that are distinct from each other, the possible identification of targets for pharmacological modulation of one or more key interactions would be promising in halting the “domino effect” of descent to valvular stenosis and heart failure.

1.13 Overview of selected biomarkers

The previous two sections have summarised the major cellular mechanisms responsible for the instigation and propagation of AS, highlighting that although these processes in isolation have been validated at a cellular level, crucial points of intersection between different phases have been difficult to ascertain. Hence, a definitive cascade of aortic valve calcification cannot yet be modelled. From a clinical standpoint, the severity and chronicity of factors such as type 2 diabetes mellitus and increased body mass index negatively influence surgical outcomes and non-operative morbidity, as highlighted at the beginning of this chapter. It is therefore prudent to interrogate these processes in the most likely chronological order as validated by previous studies, beginning at the initiation phase, in particular the lipid infiltration phase (Figure 9).

The review of the literature presented in this chapter has provided focus and direction in the selection of the biomarkers selection for this study, which will focus on lipid deposition. These biomarkers have already been alluded to in the prior section, however a brief introduction of the six biomarkers of lipid metabolism that were assessed as part of the second study (Chapter 4) is presented here. In some cases, controversy still remains as to the exact relationship of some markers with AS or T2DM, necessitating their inclusion in the hope of providing additional clarity as to their roles. These include three major adipokines and three major lipoprotein which have been validated to play a role in both cardiovascular atheroma formation and calcification, as well as insulin resistance.

1.13.1 Adiponectin

Adiponectin is a fat-derived hormone which functions as an important messenger in the crosstalk between adipose tissue and other metabolically active organs. It targets the liver, heart, beta islet cells of the pancreas, kidney and muscle amongst other organs and tissues (131). The actions of adiponectin include suppression of gluconeogenesis by the liver at a genetic level, improves insulin sensitization of cells and provides resilience towards physiological and pathological mechanisms that cause cellular apoptosis (132).

In the context of cardiovascular disease there is focused interest in the role of adiponectin not only due to its observed cardioprotective association with increased circulating levels, but also for the possible local, almost paracrine-like action on coronary arteries and heart valves, as it is produced in small amounts by adjacent cardiomyocytes (133). The inverse relationship between levels of circulating adiponectin and body mass index is well established, and this correlation has also been found to hold true with the incidence in severity of coronary artery disease, however there is conflicting evidence of its role in isolated aortic valve stenosis (134). Conversely, the correlation of decreased adiponectin levels in the presence of type 2 diabetes mellitus is well established, therefore the inclusion of adiponectin in this study will attempt to elucidate if increased levels of adiponectin in people without type 2 diabetes mellitus or the metabolic syndrome correlates with a reduced disease burden by objective echocardiographic criteria (135).

1.13.2 Leptin

Leptin is a pleiotropic hormone which regulates many physiological processes pertaining to appetite, thermogenesis, cardiovascular regulation, haemostasis, and immune function. Similar to adiponectin, leptin is predominantly secreted by adipose tissue and is also secreted from other tissues, including the heart, via autocrine or paracrine effects (136). Leptin regulates appetite by controlling satiety signals to the central nervous system (CNS) and it influences cardiovascular functions either directly or indirectly via secondary responses mediated by the vasculature (such as hypertension, endothelial function, atherosclerosis, and thrombopoiesis) or the CNS.

The cumulative effect of leptin levels on the cardiovascular system is negative, however this relationship is complex. Leptin deficiency has both and obesogenic effect and is deleterious to myocardial and vessel wall (137). In contrast to the beneficial effects in the majority of cases, leptin, particularly in the context of obesity-associated hyperleptinemia, exerts detrimental effects in cardiovascular function and promotes adverse outcomes in cardiovascular disorders (138). Such paradoxical findings warrant further examination, and a common consensus is a theory of adipokine balance, with the adiponectin to leptin ratio currently being interrogated in various metabolic conditions as a potential marker of overall status.

In the context of aortic stenosis, recent studies have eluded to a link between leptin and disease progression at a valvular level, evidenced by altered haemodynamic parameters, although it remains difficult to tease out the exact role of leptin on valvular disease in isolation (139). Established large cohort studies of leptin in coronary artery disease have yielded conflicting findings, suggesting that this may be the case, given the complex interplay between insulin resistance and other cardiovascular and obesogenic risk factors.

1.13.3 Resistin

Resistin is a cysteine-rich peptide hormone derived from adipose tissue. It is mainly secreted by the bone marrow, monocytes, and macrophages. It contributes to many processes, including endothelial dysfunction, vascular smooth muscle cell (VSMC) proliferation, and atherothrombosis demonstrating effects on the development of hypertension, coronary artery disease and congestive heart failure. Studies have also demonstrated its involvement in peripheral artery disease (140).

The role of resistin in lipid metabolism, inflammation and insulin resistance in rodent studies is well established, however controversy remains in its precise role in humans, and a recent meta-analysis concluded that In T2DM and obese individuals, resistin levels were positively correlated with insulin resistance in those with hyperresistinemia, but not in those with normal circulating resistin levels (141).

In AS however, initial studies by Kolasa-Trela et al concluded that in contrast to adiponectin and leptin, low levels of resistin were not associated with AS (142). In contrast, several studies have now established links not only with increased valvular calcification, but also increased aortic stiffness with elevated levels of resistin. More recently, a rodent study explored the expression of resistin in aortic valves with scanning electron microscopy (SEM) and western blot, and also the effect of resistin deletion on valve cells (VICs) calcification. The results demonstrated that the deletion of resistin protected mice from developing aortic stenosis. This deletion also demonstrated a protective effect in preventing these mice from developing obesity (143).

The inclusion of resistin in the second study (Chapter 4) will help add context to the involvement of adiponectin and resistin, thus providing a more comprehensive picture of an adipokine profile in various states of disease.

1.13.4 Lipoprotein-A

Lipoproteins function to transport lipids in bodily fluids such as plasma and extracellular fluid. They consist of a central core made up of cholesterol and a triglyceride, with a peripheral arrangement of phospholipids. This construction is also the make-up of various structural proteins, enzymes, transport molecules and antigens. Lipoprotein A is a low-density lipoprotein which is well established as a risk factor for atherosclerotic and thrombosis (144). Although structurally similar to low density lipoproteins, randomised trials have shown no benefit of statin therapy on AS progression despite a marked reduction in cholesterol levels in individuals with mild to moderate AS (145). Genetic variation mediated by lipoprotein-A levels has been shown to be associated with aortic valve calcification and incident AS in multiple ethnic groups (146). In the Copenhagen General Population study, lipoprotein-A levels were correlated with progressively increased risk of AS after adjustment for risk factors including age and cholesterol. People with above 90th percentile levels had a twofold to threefold increased risk of AS (147).

Epidemiological and prospective data have suggested that high levels of lipoprotein-A is an independent risk factor for incident cardiovascular disease, particularly among those with T2DM. In observational data, lower levels of lipoprotein-A have been associated with greater prevalence of type 2 diabetes (148). This paradoxical relationship would imply that elevated lipoprotein-A levels is a precursor to cardiovascular disease in people with T2DM, thus a potential role as a biomarker would be feasible. It is not clear whether targeted therapeutic approaches of lowering lipoprotein-A levels could result in delaying the onset or preventing the development of cardiovascular diseases in these patients.

1.13.5 Apolipoproteins A1 and B

Apolipoproteins are the major protein component of lipoproteins which fulfil the role of transporting lipids. They and are important in maintaining the structural integrity and solubility of lipoproteins. Apolipoproteins play a role in lipoprotein receptor recognition and in the regulation of certain enzymes in lipoprotein metabolism (149).

Apolipoprotein A1 is the major structural and functional protein component of high density lipoprotein (HDL) in plasma, constituting approximately 70% of HDL. It is the primary protein constituent of HDL that defines its size and shape, solubilizing its lipid component, and aids reverse cholesterol transport. Being a cofactor for lecithin cholesterol acyl transferase (LCAT), for the formation of most plasma cholesteryl esters, it promotes cholesterol efflux from tissues to the liver for excretion (150).

Apolipoprotein B (Apo B) is a major protein component of low-density lipoprotein (LDL) comprising >90% of the LDL proteins and constituting 20-25% of the total weight of LDL. Increased plasma concentration of Apo B-containing lipoproteins is associated with an increased risk of developing atherosclerotic disease. Case control studies have found plasma Apo B concentrations to be more discriminating than other plasma lipids and lipoproteins in identifying patients with coronary heart disease (CHD). Apo B measurement offers greater precision than LDL cholesterol determination which is most often derived by calculation (151).

The inclusion of these two biomarkers in this thesis aim to further scrutinize the effect of serum cholesterol in the participants, as the 2019 joint consensus statement by the European Society of Cardiology/European Atherosclerosis Society stated that apolipoprotein B was a more accurate marker of cardiovascular risk than low-density lipoprotein cholesterol.

1.14 Background and brief overview of project design

Type 2 diabetes mellitus (T2DM) has been established as a risk factor for aortic stenosis (AS), however evidence of accelerated progression in people with AS with T2DM (i.e. earlier symptomatic presentation, increased underlying valve calcification and increased incidence of left ventricular (LV) remodelling) compared to people with AS without T2DM is limited. Evidence for the impact of metabolic syndrome on people with severe AS is lacking.

The need to ascertain if T2DM or metabolic syndrome (MetS) induce subclinical progression of AS within a population is important as current guidelines on aortic valve intervention (i.e. aortic valve replacement, AVR or transcatheter aortic valve implantation, TAVI) emphasizes the symptomatic status and echocardiographic findings of haemodynamic compromise rather than the underlying burden of disease. LV remodelling (which occurs either as a consequence of chronic pressure overload or inflammatory changes secondary to hyperglycaemia or insulin signalling) may be more amenable to reversal from early intervention. Thus, a method of identifying people who are likely to have a more advanced underlying disease state in the absence of symptoms (or those with only mild symptoms) irrespective of echocardiographic findings is warranted as early intervention on such people will improve long-term cardiovascular outcomes.

Cellular mechanisms for aortic valve calcification are now accepted to be distinct from those for arterial calcification. Although there are various processes in play resulting in valve calcification, a common link between the pathogenesis of aortic valve calcification and diabetic cardiomyopathy is lipid infiltration triggering increased cytokine activity resulting in chronic inflammation. Following a review of literature highlighting the role of lipids and adipokines in aortic valve calcification (particularly in the initiation phase) and their associations with myocardial remodelling, this study has interrogated several key areas in which evidence is lacking or absent in a cohort of people with AS and T2DM.

This study has endeavoured to firstly establish whether there is a difference in the structural and haemodynamic burden on both the aortic valve and myocardium in people with MetS/DM with severe AS. Secondly, this study assesses whether these pre-existing disease states correlate with any postoperative improvement of left ventricular function. Finally, the correlation of selected serum markers of lipid activity with preoperative disease burden will be evaluated.

1.15 Hypothesis

The null hypotheses of this study are presented below

a) In a population of people in Wessex undergoing surgery for isolated AVR for severe AS, there is no difference in the echocardiographic characteristics of valve and ventricular function burden between those with and without MetS/T2DM.
b) Following an interval after AVR in this population, any evidence of LV remodelling by echocardiography is independent of preexisting MetS/T2DM
c) In a population of people in Wessex undergoing surgery for isolated AVR for severe AS, there is no difference in baseline levels of serum markers of lipid metabolism in people with and without MetS/T2DM.
d) In this same population of people, these levels of serum markers of general inflammatory activity do not correlate with preoperative echocardiographic parameters of aortic valve disease severity or ventricular function.

Chapter 2. Participants and General Methods

2.1 Ethical Approval and Participant Recruitment

This was a single-centre study at a secondary care cardiothoracic surgical unit in Wessex. I personally sought ethical approval and following a review by the Hampshire B National Research Ethics Committee, the study was deemed to follow all principles set out by the National Institute of Health and Care Research (NIHR) Good Clinical Practice clinical and research guidance, and thus ethical approval was granted by the Hampshire B National Research Ethics Committee (NRES) – South Central (REC Ref: 18/SC/0162) (Appendix, page 144) University Hospital Southampton NHS Foundation Trust Research and Development department acted as the local sponsor (IRAS ID: 240397, Ref: RHM CAR 0541) (Appendix, page 137). For the prospective study cohort, people admitted electively and urgently to University Hospital Southampton for aortic valve replacement surgery were approached and invited to participate. Prospective participants were counselled face-to-face by me and received dedicated patient information sheets (Appendix, page 147) prior to providing verbal and written consent (Appendix, page 152) confirming participation. Confirmation of ethical approval, the patient information leaflet and the consent form are included here.

Absolute inclusion and exclusion criteria declared in the study protocol are as follows:

Inclusion Criteria:

a) Age (years): 40 - 90 years old
b) Gender: All
c) People undergoing first-time aortic valve replacement for aortic stenosis.
d) People with and without diabetes

Exclusion Criteria:

a) Age (years) < 40 years old or > 90 years old
b) Active infective endocarditis
c) Redo Aortic Valve Surgery
d) Active Cancer
e) Inflammatory conditions which may affect the results of blood biomarkers
f) People on renal replacement therapy
g) Emergency surgery – as decided by the Consultant Surgeon

Following the commencement of recruitment for this study, several other criteria have been included as **relative exclusion** criteria (due to practical challenges in conducting patient follow-up)

a) Transport/mobility/home circumstances – these are of particular relevance with people of more advanced age, and hence may have to be excluded on a case-by-case basis if follow-up attendance may be inconvenient to the individual.
b) Channel Islanders – UHS is the sole cardiac surgery centre for people from the islands of Jersey and Guernsey. Follow-up attendance would require air transport and an overnight stay, which we are not able to provide given our study budget.

2.2 Sample size

The cross-sectional and observational nature of the studies undertaken present a challenge in providing a truly representative sample size calculation, however this has been undertaken for the sake of completeness.

The total percentage prevalence of diabetes in the UK is quoted as 6%. Large epidemiological studies estimate the prevalence of diabetes in patients with severe aortic stenosis to be between 14.4% and 19.7%. To minimize the likelihood of misrepresenting the effect of diabetes, we will utilise a prevalence percentage of 20% and a confidence interval of 99%.

Sample size calculation has been done as follows:

Confidence interval, c = 95% (0.95 for calculation purposes)

Power of study = 80%

Estimated proportion of severe AS patients without diabetes, p1 = 0.8

Estimated proportion of severe AS patients with diabetes, p2 = 0.2

Critical value of normal distribution at $\alpha/2$, $Z\alpha/2 = 1.96$

Critical value of normal distribution at β, $Z\beta = 0.84$

By applying the formula as described in Wang, H. and Chow, S.-C. 2007. Sample Size Calculation for Comparing Proportions, the sample size (n) is as follows

$n = (Z\alpha/2 + Z\beta)2 * [p1 (1 - p1) + p2 (1 - p2)] / (p1 - p2)2$

$n = (1.96 + 0.84)2 * [0.8 (1 – 0.8) + 0.2 (1 – 0.2)] / (0.8 – 0.2)2$

$n = 7.84 \times 0.32 / 0.36$

$n = 6.99 \approx 7$

A minimum of 7 AS patients in each group (Control, MetS and T2DM) is required to achieve a statistical power of 0.8 with a confidence interval of 95%. Therefore, a minimum of 21 patients in total would achieve this. The same calculation performed to achieve a statistical power of 0.8 with a confidence interval of 99% is a minimum of 11 patients in each group (a minimum of 33 patients in total). To account for the possibility of attrition at follow-up, we have recruited 42 patients.

2.3 Baseline demographic data

I collated baseline demographic data on all the study participants via the UHS electronic patient database system (ICE), which provides access to all patient correspondence (i.e. referral and clinic letters), past medical history and blood test results. Relevant data for this study is age, height, weight, body mass index, hypertension (blood pressure ≥140/90 mmHg or on antihypertensive medication), breathlessness status (via the NYHA classification), angina status (via the CCS classification), presence of T2DM, hypercholesterolaemia, smoking status, and current prescription medications. Results from preoperative blood tests of interest are fasting plasma glucose, glycated haemoglobin (HbA1C), total cholesterol, triglycerides, HDL, LDL, C-reactive protein (CRP), neutrophil count, lymphocyte count and platelet count.

2.4 Preoperative Transthoracic Echocardiography Protocol

As patients are routinely referred following an abbreviated screening echocardiogram, all study participants underwent full valvular and ventricular echocardiographic assessment performed prior to surgery. I undertook a review of all these echocardiogram studies and tabulated the relevant parameters. All study echocardiography was performed using an iE33 ultrasound equipped with an S3 sector array probe (Phillips Medical Systems, Best, The Netherlands). Two-dimensionally guided M-mode echocardiograms were performed on each subject and all measurements and calculations were done using the following protocol:

a) M-mode measurements

All M-mode tracings were obtained at 50 mm/s. Measurements of left ventricular end-diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), interventricular septum thickness (IVS), posterior wall thickness (PWT) were performed according to the guidelines of the American Society of Echocardiography. Left ventricular mass (LVM) was calculated according to the formula

$$LVM\ (g) = 0.8 \times \{1.04 \times [(LVEDD + IVS + PWT)^3\ 2\ LVEDD^3]\} + 0.6$$

Left ventricular mass was indexed (LVMi) for body height in metres, normalized to the allometric power of 2.7, which linearizes the relations between LVM and height and identifies the impact of obesity. Left ventricular hypertrophy was defined as an LVMi >44 g/$m^{2.7}$ for women and >48 g/$m^{2.7}$ for men. Left ventricular end-diastolic and end-systolic volumes (LVEDV, LVESV) were determined using the Teichholz equations:

$$LVEDV\ (mL) = [7/(2.4 + LVEDD)] \times LVEDD^3 \text{ and } LVESV\ (mL)\ ¼\ [7/(2.4 + LVESD)] \times LVESD^3$$

The ejection fraction was calculated as EF = (LVEDV - LVESV)/LVEDV

b) Two-dimensional measurements

The diameter of the left ventricular outflow tract (LVOT) was evaluated in zoomed apical five-chamber view. Aortic valves were scanned from the parasternal short-axis and the apical five-chamber view. Degenerative aortic valve disease was characterized by an abnormal irregular thickening or a focal or diffuse increase of the echogenicity of the leaflets with or without reduced systolic opening.

c) Doppler measurement

All Doppler echocardiographic recordings were registered with 100 mm/s and performed in expiration. Velocity time integrals of flow from the LVOT and from the aortic valve were evaluated using pulsed continuous wave Doppler. Using the continuity equation, aortic valve area (AVA) was calculated as:

$$AVA\ (cm^2) = (VTI_{LVOT}/VTI_{AV}) \times (0.5 \times LVOT)^2 \times \phi$$

where ϕ = velocity potential.

2.5 Sample acquisition and storage

I undertook arterial blood sampling on the study participants on the day of surgery following the insertion of an arterial line (as a routine part of cardiac surgery). 10ml of blood was initially drawn and discarded (to prime the arterial catheter) and a further 12ml of blood collected at normal body temperature. These were immediately decanted into six 2ml cryovials and transferred to the lab in under one hour.

2.6 Preparation of serum samples

a) Whole blood samples in cryovials were submerged in a water bath at 37°C
b) The cryovials were then manually agitated by hand for 60 seconds
c) The exterior of the vials were cleaned by wiping it with 70% ethanol
d) The cryovials were then opened and sample pipetted into a plain centrifuge tube
e) The samples were centrifuged at 30000rpm for 10 minutes at 4°C
f) Immediately after centrifugation, the spun serum was pipetted into cryovials snap-frozen with liquid nitrogen and stored at -70°C

2.7 Assessment of serum samples

Aliquots of serum samples were removed from the deep freezer, packed in dry ice sent to the Core Biochemical Assay Laboratory (CBAL; Cambridge, UK) for analysis. Transport time was under 2 hours, and no samples were compromised. The protocol below is provided by CBAL as to the method used:

Serum cytokines were measured using a commercially available 10-plex electrochemical luminescence immunoassay (V-Plex 10-plex human proinflammatory cytokine kit; MesoScale Discovery, Gaithersburg, Maryland, USA) on a MesoScale Discovery Sector 6000 analyser. All reagents and calibrators were supplied with the kit and the assay protocol was set up according to manufacturer's instructions.

Insulin was measured using a MesoScale Discovery kit (Gaithersburg, Maryland, USA). Plates were read on an MSD Sector 6000 plate reader. Triglyceride concentration was measured using a commercially available colourimetric enzymatic assay (Siemens Healthcare, Germany), with absorbance measured on a Siemens Dimension RxL analyser. NEFAs were measured using a commercially available Roche Free Fatty Acid kit run on a microtitre plate.

CRP was measured using an in-house Dissociation-Enhanced Lanthanide Fluorescent Immunoassay (DELFIA) protocol specific to CBAL, but using reagents from an R&D systems DuoSet assay kit (Minneapolis, USA). Data was recorded on a Perkin Elmer Victor3 time-resolved fluorescence plate reader (Massachusetts, USA).

2.8 Statistical Analysis

Continuous data was presented as a mean ± standard deviation and categorical data as prevalence (%). The Kolmogorov–Smirnov test was performed to assess conformity with normal distribution.

Unpaired categorical variables were compared by the Fisher exact test or $\chi 2$ test as appropriate.

The t-test and one-way analysis of variance (ANOVA) were applied to determine differences between continuous variables. A linear regression analysis was used to correlate variables. An analysis of covariance (ANCOVA) was performed to eliminate the confounding effect of age and body mass index (BMI) on tested variables among patients with different AS severity. The ANOVA with post-hoc Sidak tests was performed to assess differences between groups.

A multiple linear regression analysis was performed in a two-step sequential model to determine the incremental value of echocardiographic (second step) over clinical (first step) predictors for adiponectin, apolipoprotein A1, apolipoprotein B, lipoprotein-A, leptin and resistin. Clinical and echocardiographic variables, which demonstrate any associations in initial linear regression analysis, was included in the sequential models. The incremental prognostic value was defined by a significant increase in the global $\chi 2$.

A univariate-logistic regression analysis was performed to determine potential predictors of severe AS among clinical, laboratory, and echocardiographic data. Afterwards, a multivariable logistic regression of these potential predictors was performed in a stepwise forward fashion to identify parameters associated with severe AS. The odds ratio (OR) with the corresponding 95% confidence interval (CI) was calculated for each parameter. Entry was set at $P < 0.05$, while retention at $P < 0.10$. $P < 0.05$ was considered statistically significant.

Chapter 3. Left Ventricular Remodelling Following Isolated Aortic Valve Replacement for Aortic Stenosis in Patients with the Metabolic Syndrome and Type 2 Diabetes

3.1 Introduction

Aortic Stenosis (AS) remains the most prevalent degenerative valve condition in the developed world, accounting for 43% of all valvular heart disease (121). In the United Kingdom, AS currently affects 1.5 million people over the age of 65 and is expected to rise to 3.3 million people by the year 2050 (49). AS is the narrowing of the aortic valve leading to impedance of efficient ejection of blood from the left ventricle (LV) and chronic pressure overload. This results in left ventricular hypertrophy (LVH) - the increase in the size of the ventricle to maintain adequate cardiac output (CO) - which is initially compensatory but ultimately maladaptive and associated with increased mortality. Surgical aortic valve replacement (AVR) or transcatheter aortic valve replacement (TAVI) remain the only definitive therapeutic options, however current guidelines for both indication and urgency of treatment centre around symptoms and narrow echocardiographic criteria which are specific to assessing compromise of cardiac output as indications for intervention (122-125).

There is emerging evidence that AS patients with type 2 diabetes mellitus (T2DM) exhibit more pronounced volumetric changes of the left ventricle compared with their non-diabetic counterparts. This is likely to have developed subclinically over a protracted length of time prior to the onset of symptoms. The exact nature of the relationship between T2DM and changes in cardiac structure and performance is unclear and remains an area of active research. The term metabolic syndrome (MetS) refers to a confluence of several risk factors for cardiovascular disease, namely insulin resistance, hypertension, dyslipidaemia, and obesity. The recognition of an AS patient population

exhibiting these factors in the absence of T2DM is important as they may be at risk of the same latent changes in LV geometry experienced by AS patients with T2DM (126-130).

3.2 Materials and Methods

An analysis of prospectively-collected data at University Hospital Southampton was conducted over a six-year period. During this timeframe, 709 patients underwent isolated AVR (i.e. AVR without any other concomitant coronary artery or valve procedures) at the University of Southampton Wessex Cardiothoracic Unit. Exclusion criteria were:

1. LV ejection fraction <50%

2. Bicuspid native aortic valve

3. Previous aortic valve surgery

4. surgery for infective endocarditis

5. aortic regurgitation > mild

6. aortic root enlargement or replacement procedures

7. lack of follow-up echocardiogram data.

Following screening for exclusion criteria, 367 patients were analysed. Approval to conduct the study was granted by the local and national ethics committees.

3.2.1 Preoperative demographic and clinical data

Demographic and clinical data were obtained from prospectively collected entries in both paper and electronic institutional records. The following categorical and continuous variables were ascertained; age, gender, height, weight, body mass index (BMI), body surface area (BSA), New York Heart Association Functional Classification for breathlessness (NYHA), Canadian Cardiovascular Society grading of angina pectoris (CCS), European System for Cardiac Operative Risk Evaluation (EuroSCORE), logistic EuroSCORE, hypertension (blood pressure ≥ 140/90 mmHg or on antihypertensive medication), extracardiac arteriopathy (carotid, aortoiliac, abdominal or peripheral arterial disease) and smoking status. Biochemical data included serum cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), glycated haemoglobin A1C (HbA1c) and fasting serum glucose. Patients were deemed positive for MetS if they exhibited any three of the following criteria proposed by the National Cholesterol Education Program - Adult Treatment Panel III, which are:

1. Blood pressure ≥ 130/85 mmHg or receiving drug therapy for hypertension

2. Fasting serum glucose ≥ 5.6 mmol/L

3. serum triglyceride ≥ 1.695 mmol/L

4. serum HDL < 1.04 mmol/L in men and < 1.30 mmol/L in women.

The fifth criterion (waist circumference of ≥ 102 cm in men and ≥ 88 cm in women) was not included in this study as this data was unavailable.

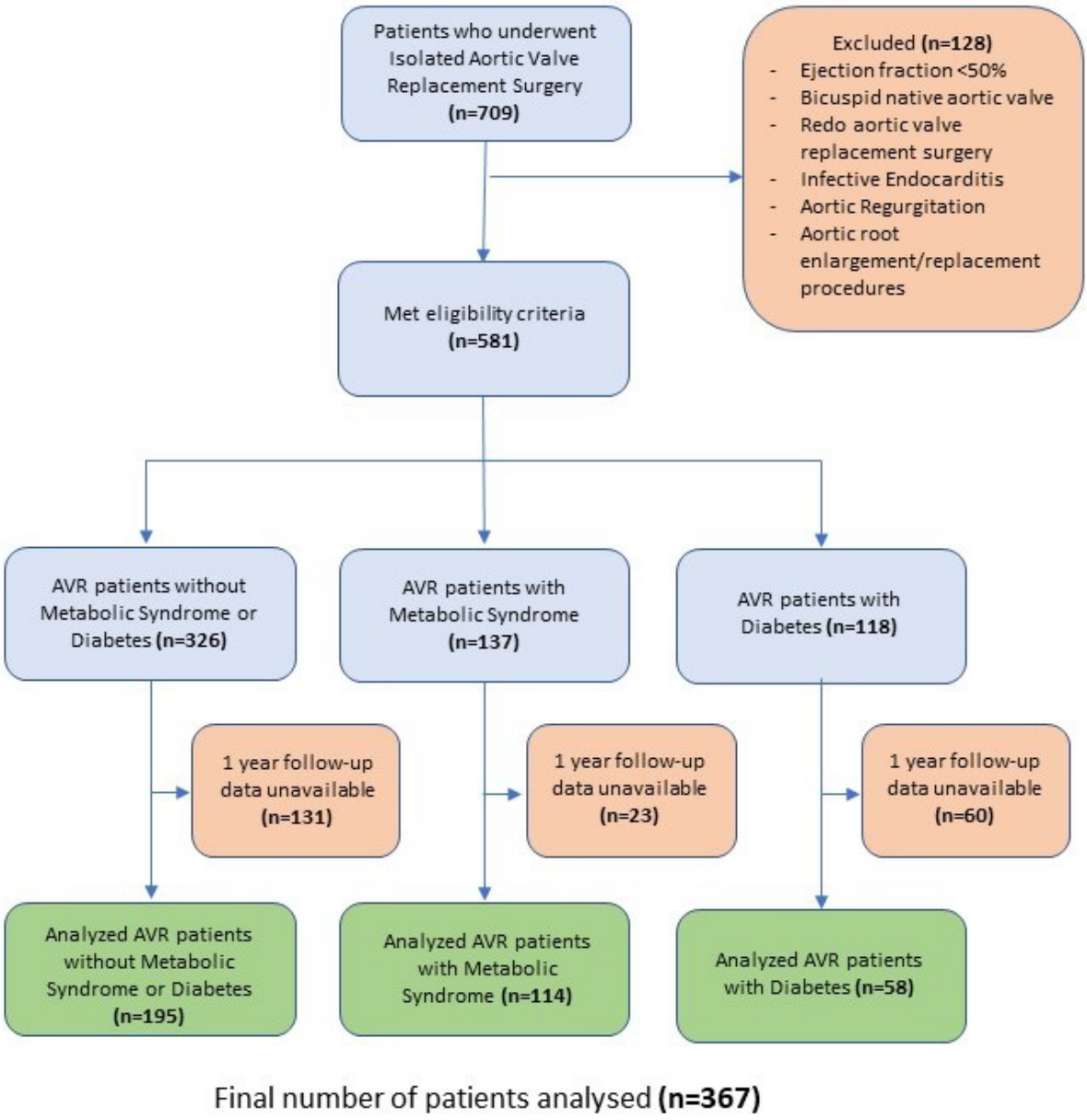


Figure 12. Flow diagram illustrating inclusion and exclusion criteria of patients analysed. Isolated aortic valve replacement refers to AVR without concomitant procedures on coronary arteries or other heart valves. AVR; aortic valve replacement

3.2.2 Echocardiography studies

All patients underwent comprehensive two-dimensional echocardiography prior to and one year following AVR which were stored in an electronic database: these were reviewed, and additional calculations and measurements were undertaken. All studies were performed using an iE33 ultrasound platform equipped with aS3 sector array probe (Philips Medical Systems, Best, Netherlands) and the following parameters were measured or calculated; ejection fraction (EF), stroke volume index, peak systolic gradient across the aortic valve, mean systolic gradient across the aortic valve, aortic valve area (and subsequent indices by height and BSA), left atrial diameter, left ventricular end systolic diameter (LVESD), left ventricular end diastolic diameter (LVEDD), interventricular septum diameter (IVS), posterior wall thickness (PWT), left ventricular mass (and subsequently indexed for height), incidence of LV hypertrophy, relative wall thickness ration (RWT) and the ratio of left ventricular mass to left ventricular end diastolic volume (LVM/LVEDV). Stroke volume was calculated by multiplying the flow velocity-time integral by the LV outflow tract area. Left ventricular mass was calculated using the modified cube formula and indexed to body surface area and to a 2.7 power of height. Left ventricular hypertrophy was defined as an indexed LV mass > 49 $g/m^{2.7}$ in men and > 47 $g/m^{2.7}$ in women. The relative wall thickness ratio was calculated as the ratio of 2 times the posterior wall thickness to LV internal diameter in diastole. By taking into account both values of LV mass and relative wall thickness ratio, patients were classified into 4 different patterns:

1. Normal: absence of LV hypertrophy and ratio ≤ 0.42
2. Eccentric hypertrophy: presence of LV hypertrophy and ratio ≤ 0.42
3. Concentric remodelling: absence of LV hypertrophy and ratio > 0.42
4. Concentric hypertrophy: presence of LV hypertrophy and ratio >0.42

Left ventricular mass regression was calculated by subtracting preoperative from postoperative indexed LV mass. The index of LV mass to end diastolic volume (mass-to-volume ratio), an alternative index of LV remodelling, was measured before and after AVR.

3.2.3 Statistical Analysis

Data were analysed using SPSS software (version 26; IBM, USA). Data were tested for normal distribution using the Shapiro-Wilk test. Continuous data were presented as mean ± SD, median were compared using paired and unpaired Student's t-test, ANOVA, or a Wilcoxon rank sum test. Categorical data were expressed as a percentage and compared using Fisher's test. Paired statistical tests were used to compare changes before and after aortic valve replacement. Post-hoc analysis for multiple groups was done using either the Holm-Sidak or Kruskal-Wallis tests as appropriate.

3.3 Results

Table 4. Baseline demographic and laboratory data

Variable (N = 367)	Control n=195 (53.1%)	MetS n=114 (31.1%)	T2DM n=58 (15.8%)	p value
Age, years	70 ± 12.7 ɣ	71 ± 9.6 ɣ	74 ± 8.3 α, β	**<0.01**
Height, m	1.67 ± 0.1	1.65 ± 0.1	1.65 ± 0.1	0.25
Weight, kg	80 ± 18.3 ɣ	79.1 ± 16.4 ɣ	88 ± 18 α, β	**<0.01**
Male gender, n	86 (44.1%)	44 (38.6%)	27 (46.6%)	0.69
BMI, kg m^{-2}	28.7 ± 6.0 ɣ	28.9 ± 5.3 ɣ	32.4 ± 6.2 α, β	**<0.01**
BSA, m^2	1.91 ± 0.25 ɣ	1.90 ± 0.23 ɣ	2.00 ± 0.29 α, β	**0.028**
NYHA				
Class I	120 (61.5%)	18 (15.7%)	7 (12.1%)	
Class II	39 (20%)	58 (50.9%)	24 (41.4%)	
Class III	31(15.9%)	37 (32.5%)	26 (44.8%)	
Class IV	5 (2.6%)	1 (0.9%)	1(1.7%)	
CCS				
Class I	34 (17.4%)	76 (66.7%)	28 (48.3%)	
Class II	108 (55.4%)	23 (20.2%)	14 (24.1%)	
Class III	45 (23.1)	13 (11.3%)	15 (25.9%)	
Class IV	8 (4.1%)	2 (1.8%)	1 (1.7%)	
EuroSCORE	6.13 ± 3 β,ɣ	6.9 ± 2.1 α	6.6 ± 2.6 α	**0.04**
Logistic EuroSCORE	8.21 ± 8.5	8.5 ± 6.6	8.4 ± 9.9	0.96
Hypertension	117 (60%)	75 (65.8%)	51 (88%)	0.07
Current/Ex-smoker	93 (47.7%)	51 (44.7%)	32 (55.2%)	0.64
Extracardiac Arteriopathy	7 (3.6%)	3 (2.6%)	6 (10.3%)	0.055
Creatinine, µmol/L	88.7 ± 58.1 α	93.4 ± 48.4 α	116.3 ± 94.7 α, β	**0.009**
Total Cholesterol, mmol/L	5.30 ± 1.46 β, ɣ	4.40 ± 1.39 α	4.27 ± 0.87 α	**<0.01**
Triglycerides, mmol/L	1.31 ± 0.54 ɣ	1.34 ± 0.33 ɣ	1.77 ± 0.97 α, β	**<0.01**
HDL, mmol/L	1.35 ± 0.42	1.39 ± 0.53	1.36 ± 0.34	0.52
LDL, mmol/L	3.95 ± 1.5 β, ɣ	3.01 ± 1.44 α	2.91 ± 0.95 α	**<0.01**
Cholesterol/HDL ratio	4.43 ± 2.45 β, ɣ	4.01 ± 2.95 α	3.37 ± 1.19 α	**0.031**
Fasting blood glucose, mmol/L	5.53 ± 0.67 ɣ	5.72 ± 1.17 ɣ	9.16 ± 3.89 α, β	**<0.01**
HbA1C, mmol/mol	32 ± 15 β, ɣ	38 ± 11 α, ɣ	59 ± 15 α, β	**<0.01**

Data presented as mean ± SD or n (%). P-values obtained from one-way ANOVA and post-hoc Holm-Sidak or Kruskal-Wallis correction as appropriate (α = $p < 0.05$ compared with control; β = $p < 0.05$ compared with MetS; ɣ = $p < 0.05$ compared with T2DM). BMI, body mass index; BSA, body surface area; NYHA, New York Heart Association functional class of breathlessness; CCS, Canadian Cardiovascular Society grading of angina pectoris; EuroSCORE, European System for Cardiac Operating Risk Evaluation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1C, glycated haemoglobin A1C.*Hypertension = blood pressure ≥140/90 mmHg or on antihypertensive medication; extracardiac arteriopathy = carotid, aortoiliac, abdominal or peripheral arterial disease.

Baseline clinical data are listed in Table 4. After accounting for exclusions, 367 patients were divided into three groups for analysis; AS patients without MetS or T2DM (Control) = 195, 53.1%; AS patients with MetS = 114, 31.1%; and AS patients with confirmed T2DM = 58, 15.8%. T2DM patients demonstrated significant differences in age (74 ± 8.3 years, $p<0.01$), weight (88 ± 18kg, $p<0.01$), BMI (32.4 ± 6.2 kg m^{-2}, $p<0.01$), and BSA (2.00 ± 0.29 m^2, $p=0.028$) compared to the other two groups. No significant difference in gender ($p=0.69$) or height ($p=0.25$) was observed between the three groups. Mean EuroSCORE (6.13 ± 3, $p=0.04$) but not the Logistic EuroSCORE (8.21 ± 8.5, $p=0.96$) was significantly lower in the control group. Compared to the other two groups, T2DM patients had significantly elevated serum creatinine (116.3 μmol/L ± 94.7, $p<0.01$), fasting blood glucose (9.16mmol/L ± 3.89, $p<0.01$) and HbA1C (59mmol/mol ± 15, $p<0.01$) but lower serum triglycerides (1.77mmol/L ± 0.97, $p<0.01$). The control group had significantly elevated total cholesterol (5.30mmol/L ± 1.46, $p<0.01$) cholesterol to HDL ratio (4.43 ± 2.45, $p=0.031$) and LDL (3.95mmol/L ± 1.5, $p<0.01$) compared to the other two groups.

Table 5. Preoperative and 1-year postoperative 2-dimensional echocardiography measurements.

Variable N = 367	Control n=195 (53.1%)	MetS n=114 (31.1%)	T2DM n=58 (15.8%)	p value
2-D Echocardiographic Parameters (Preoperative)				
Ejection fraction, %	65 ± 9	67 ± 8	67 ± 11	0.68
Stroke volume index, ml/m$^{2.04}$	29 ± 9 $^{\beta, \gamma}$	26 ± 5 $^{\alpha}$	26 ± 7 $^{\alpha}$	**<0.01**
Peak gradient, mmHg	74 ± 19	77 ± 20	76 ± 29	0.47
Mean gradient, mmHg	46 ± 16	48 ± 19	46 ± 15	0.24
Aortic Valve Area, cm^2	0.72 ± 0.2	0.75 ± 0.16	0.74 ± 0.11	0.18
AVA indexed by BSA, cm^2/m^2	0.7 ± 0.22	0.71 ± 0.13	0.7 ± 0.17	0.51
AVA indexed by height, cm^2/m$^{2.04}$	0.4 ± 0.06	0.41 ± 0.12	0.41 ± 0.09	0.29
Left Atrial Diameter, cm	0.35 ± 0.07 $^{\beta, \gamma}$	0.39 ± 0.02 $^{\alpha, \gamma}$	0.42 ± 0.05 $^{\alpha, \beta}$	**0.02**
LVESD, cm	2.73 ± 0.31 $^{\gamma}$	2.71 ± 0.42 $^{\gamma}$	2.61 ± 0.44 $^{\alpha, \beta}$	**0.02**
LVEDD, cm	4.65 ± 0.68	4.58 ± 0.4	4.54 ± 0.41	0.35
IVS, cm	1.19 ± 0.2 $^{\beta, \gamma}$	1.31 ± 0.21 $^{\alpha, \gamma}$	1.34 ± 0.28 $^{\alpha, \beta}$	**0.03**
PWT, cm	1.07 ± 0.18 $^{\beta, \gamma}$	1.15 ± 0.16 $^{\alpha}$	1.13 ± 0.21 $^{\alpha}$	**<0.01**
LV mass, g	193 ± 49 $^{\beta, \gamma}$	216 ± 52 $^{\alpha}$	218 ± 73 $^{\alpha}$	**<0.01**
LV mass indexed by height, g/m$^{2.7}$	51.5 ± 13.2 $^{\beta, \gamma}$	56.3 ± 14 $^{\alpha}$	56.4 ± 14.6 $^{\alpha}$	**0.04**
LV Hypertrophy	99 (51%) $^{\beta}$	77 (66%) $^{\alpha}$	41 ± (60%)	**0.044**
Relative wall thickness ratio	0.47 ± 0.11 $^{\beta, \gamma}$	0.52 ± 0.08 $^{\alpha}$	0.51 ± 0.09 $^{\alpha}$	**0.037**
LVM/LVEDV, g/ml	1.98 ± 0.41 $^{\beta, \gamma}$	2.22 ± 0.33 $^{\alpha}$	2.23 ± 0.35 $^{\alpha}$	**<0.01**
2-D Echocardiographic Parameters (1-year postoperative)				
Ejection fraction, %	65 ± 6	67 ± 8	68 ± 6	0.55
Stroke volume index, ml/m$^{2.04}$	29 ± 7	27 ± 7	28 ± 5	0.45
Peak gradient, mmHg	25 ± 10	26 ± 8	26 ± 11	0.78
Mean gradient, mmHg	14 ± 7	15 ± 5	15 ± 5	0.83
Aortic Valve Area, cm^2	1.3 ± 0.41	1.35 ± 0.32	1.37 ± 0.38	0.25
AVA indexed by BSA, cm^2/m^2	0.73 ± 0.22 $^{\gamma}$	0.72 ± 0.16 $^{\gamma}$	0.79 ± 0.19 $^{\alpha, \beta}$	**0.02**
AVA indexed by height, cm^2/m$^{2.04}$	0.48 ± 0.17	0.49 ± 0.14	0.51 ± 0.2	0.61
Left Atrial Diameter, cm	0.35 ± 0.11 $^{\beta, \gamma}$	0.38 ± 0.05 $^{\alpha, \gamma}$	0.43 ± 0.09 $^{\alpha, \beta}$	**0.02**
LVESD, cm	2.71 ± 0.41 $^{\beta}$	2.69 ± 0.29 $^{\alpha, \gamma}$	2.71 ± 0.45 $^{\beta}$	**<0.01**
LVEDD, cm	4.61 ± 0.55	4.58 ± 0.47	4.53 ± 0.51	0.5
IVS, cm	1.11 ± 0.22 $^{\beta, \gamma}$	1.28 ± 0.18 $^{\alpha}$	1.27 ± 0.23 $^{\alpha}$	**<0.01**
PWT, cm	0.99 ± 0.17 $^{\beta, \gamma}$	1.05 ± 0.16 $^{\alpha}$	1.04 ± 0.2 $^{\alpha}$	**0.048**
LV mass, g	163 ± 51 $^{\beta, \gamma}$	187 ± 42 $^{\alpha}$	193 ± 65 $^{\alpha}$	**0.02**
LV mass indexed by height, g/m$^{2.7}$	39.9 ± 12.7 $^{\beta, \gamma}$	49.1 ± 14.3 $^{\alpha}$	47.4 ± 14.8 $^{\alpha}$	**0.03**
LV Hypertrophy	41 (21%) $^{\beta, \gamma}$	48 (24%) $^{\alpha, \gamma}$	33 (37%) $^{\alpha, \beta}$	**<0.01**
Relative wall thickness ratio	0.45 ± 0.11 $^{\beta, \gamma}$	0.48 ± 0.07 $^{\alpha}$	0.5 ± 0.06 $^{\alpha}$	**0.02**
LVM/LVEDV, g/ml	1.71 ± 0.4 $^{\beta, \gamma}$	1.93 ± 0.36 $^{\alpha, \gamma}$	2.11 ± 0.39 $^{\alpha, \beta}$	**<0.01**
Changes in echocardiographic parameters before and 1 year after AVR				
Absolute LV mass regression	38 ± 47	36 ± 68	32 ± 41	0.77
Indexed LV mass regression	11.2 ± 19	10.7 ± 19	8.6 ± 21	0.68
Normalization of LV hypertrophy	62 (32%)	33 (29%)	13 (23%)	0.18

Data presented as mean ± SD or n (%). P-values obtained from one-way ANOVA and post-hoc Holm-Sidak or Kruskal-Wallis correction as appropriate (α = $p < 0.05$ compared with control; β = $p < 0.05$ compared with MetS; γ = $p < 0.05$ compared with T2DM). AVA, aortic valve area; BSA, body surface area; LVESD, left ventricular end systolic diameter; LVEDD, left ventricular end diastolic diameter; IVS, interventricular septum diameter; PWT, posterior wall thickness; RWT, relative wall thickness; LV, left ventricle; LVM, left ventricular mass; LVEDV, left ventricular end diastolic volume

Table 5 summarizes differences in preoperative and one-year postoperative 2-dimensional transthoracic echocardiographic (TTE) measurements in patients who underwent isolated AVR for AS, as well as changes in LV mass and hypertrophy before and after AVR. Preoperative TTE data demonstrated significant differences in indexed stroke volume in the control group compared with the MetS and T2DM groups (29 ± 9 ml/$m^{2.04}$, $p < 0.01$). No differences in ejection fraction, peak and mean transvalvular gradients or aortic valve area (absolute and indexed for height and BSA) were observed. Compared with the MetS and T2DM groups, the control group demonstrated significant differences in the following dimensions; left ventricular end systolic diameter (LVESD = 2.73 ± 0.31cm, $p = 0.02$), posterior wall thickness (PWT = 1.07 ± 0.18cm, $p < 0.01$), absolute LV mass (LVM = 1.07 ± 0.18g, $p < 0.01$), LV mass indexed by height (LVMi = 51.5 ± 13.2g/$m^{2.7}$, $p = 0.04$), relative wall thickness ratio (RWT = 0.47 ± 0.11, $p = 0.037$) and ratio of left ventricular mass to left ventricular end diastolic volume (LVM/LVEDV = 1.98 ± 0.41g/ml, $p < 0.01$). DM patients demonstrated significantly increased LVSED compared with the other two groups (2.61 ± 0.44cm. $p = 0.02$). Significant differences between all three groups were seen with regards to left atrial diameter ($p = 0.02$) and interventricular septum diameter ($p = 0.03$). The MetS group and T2DM group showed significantly increased incidence of preoperative LV hypertrophy compared to the control group (66% MetS and 60% T2DM vs 51% Control, $p = 0.044$).

One-year postoperative TTE measurements yielded the following. Between all three groups, there was significant differences in left atrial diameter ($p = 0.02$), LV hypertrophy ($p < 0.01$) and LVM/LVEDV ration ($p < 0.01$). T2DM patients had increased AVA indexed by BSA (0.79 ± 0.19 cm^2/m^2, $p = 0.02$). MetS patients had reduced LVSED (2.69 ± 0.29cm, $p < 0.01$) compared to the control and T2DM cohort. The control group demonstrated differences in the following dimensions; IVS (1.11 ± 0.22cm, $p < 0.01$), PWT (0.99 ± 0.17cm, $p = 0.048$), LV mass (163 ± 51g, $p = 0.02$), LVMi (39.9 ± 12.7 g/$m^{2.7}$, $p = 0.03$) and RWT (0.45 ± 0.11, $p = 0.02$).

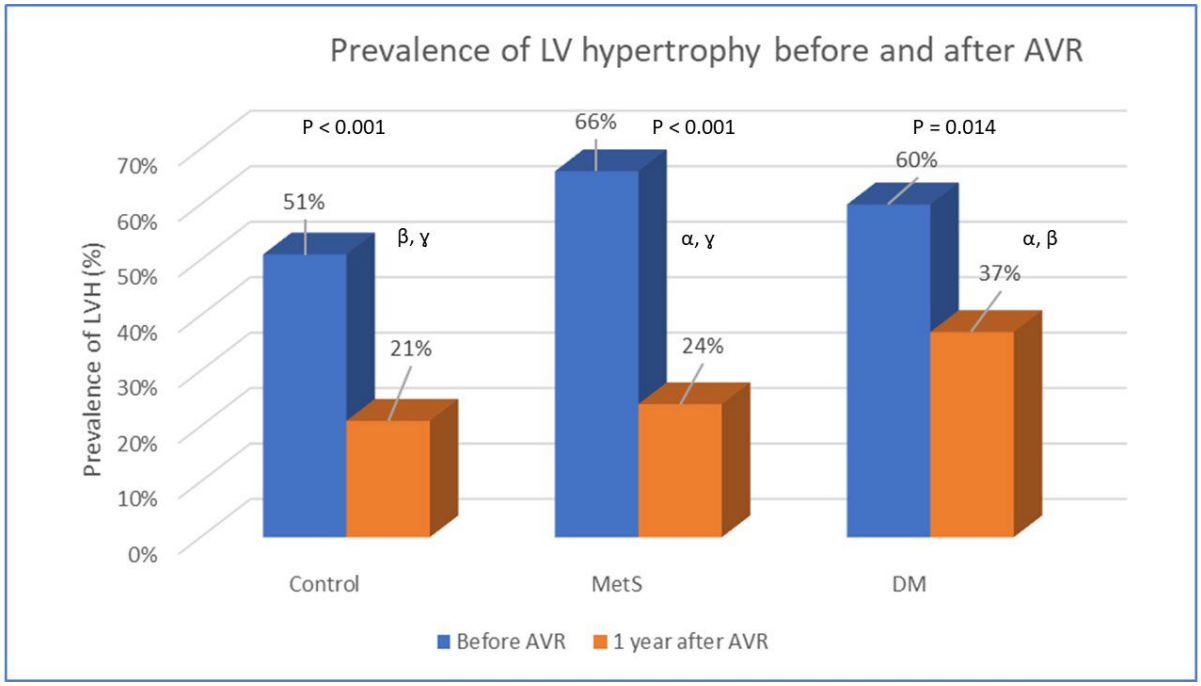


Figure 13. Prevalence of LV hypertrophy before (blue) and after (orange) AVR by percentage. AVR; aortic valve replacement, MetS; metabolic syndrome, T2DM; type 2 diabetes mellitus. P-values obtained from one-way ANOVA and post-hoc Holm-Sidak or Kruskal-Wallis correction as appropriate (α = $p < 0.05$ compared with control; β = $p < 0.05$ compared with MetS; ɣ = $p < 0.05$ compared with T2DM).

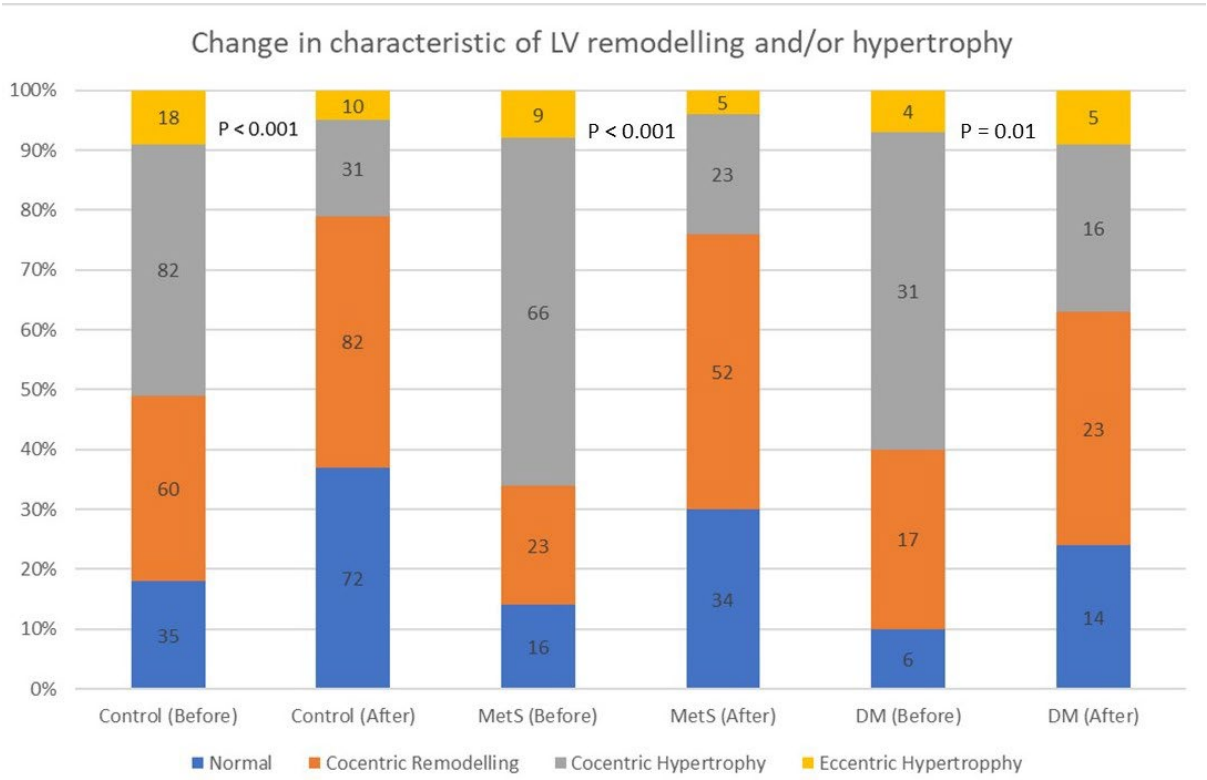


Figure 14. Bar chart rounded up to 100% of the change in left ventricular remodelling patterns before and after AVR. Blue = normal, orange = concentric remodelling, grey = concentric hypertrophy, yellow = eccentric hypertrophy. The y-axis represents cumulative percentage. Numbers within the bar chart equated to the actual number of patients. P-values are given for change within each group.

Figure 13 highlights the prevalence of LV hypertrophy before and after AVR. All three groups exhibited a reduction in LV hypertrophy which was significant (Control = 51% to 21%, $p < 0.001$, MetS = 66% to 24%, $p < 0.001$, T2DM = 60% to 37%, $p = 0.014$) however the was no significant difference between groups (Table 2, $p = 0.18$). Figure 14 further dissects the changes in LV geometry based on the four aforementioned patterns of LV remodelling or hypertrophy (i.e. normal, concentric remodelling, concentric hypertrophy and eccentric hypertrophy). Again, there was significant normalization of LV hypertrophy within each group but not between groups.

Figures 15 and 16 present the differences in change in LV mass and change in LVM/LVEDV ratio in all three groups. Reduction in LV mass was significant within each group (Control; $p < 0.001$, MetS; $p < 0.002$, T2DM; $p = 0.001$). Post-hoc analysis from 2-way ANOVA yielded a significant difference in LV mass regression between the control group compared with the MetS and T2DM groups ($p < 0.01$). All three groups demonstrated significant reduction in LVM/LVEDV ratio (Control; $p < 0.001$, MetS; $p < 0.01$, T2DM; $p = 0.013$). Post-hoc analysis from 2-way ANOVA confirmed significant differences between all three groups ($p < 0.01$).

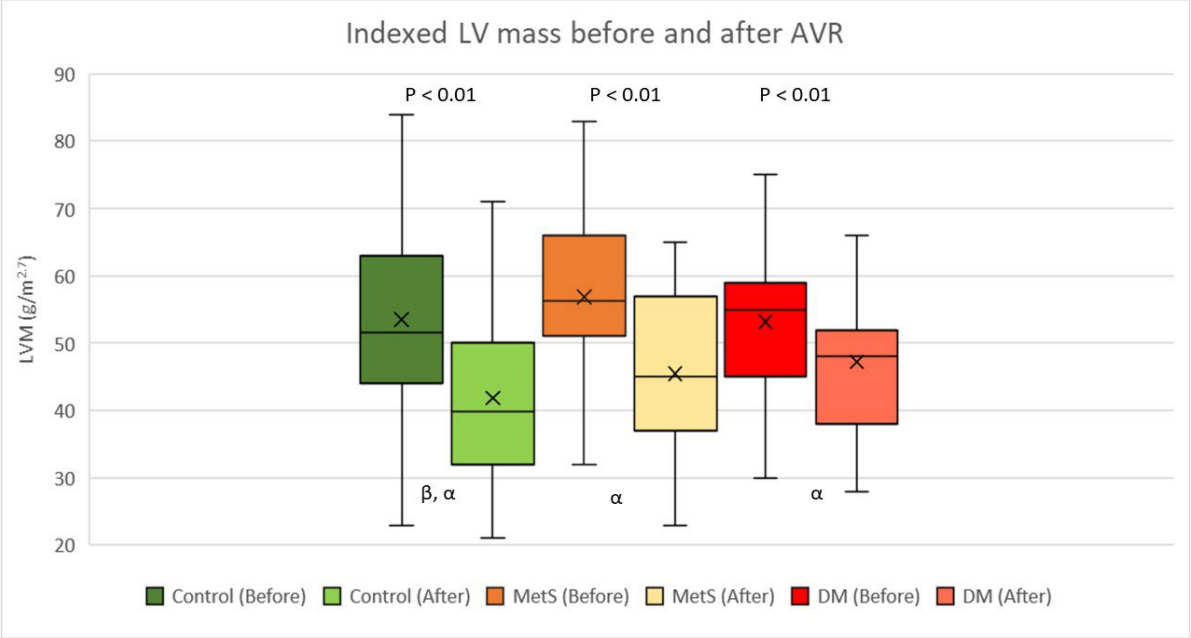


Figure 15. Box and whisker plot of left ventricular mass indexed to the power of 2.7 of the height of patients before and after AVR. Boxes are presented with median (middle line), mean (cross), 25th percentile (lower box edge) and 75th percentile (upper box edge), Whiskers represent upper and lower adjacent values. P value represents results following 2-way ANOVA. LV; left ventricle, AVR; aortic valve replacement, LVM; LV mass, MetS; metabolic syndrome, T2DM; type 2 diabetes mellitus. α = p < 0.05 compared with control; β = p < 0.05 compared with MetS; ɣ = p < 0.05 compared with T2DM

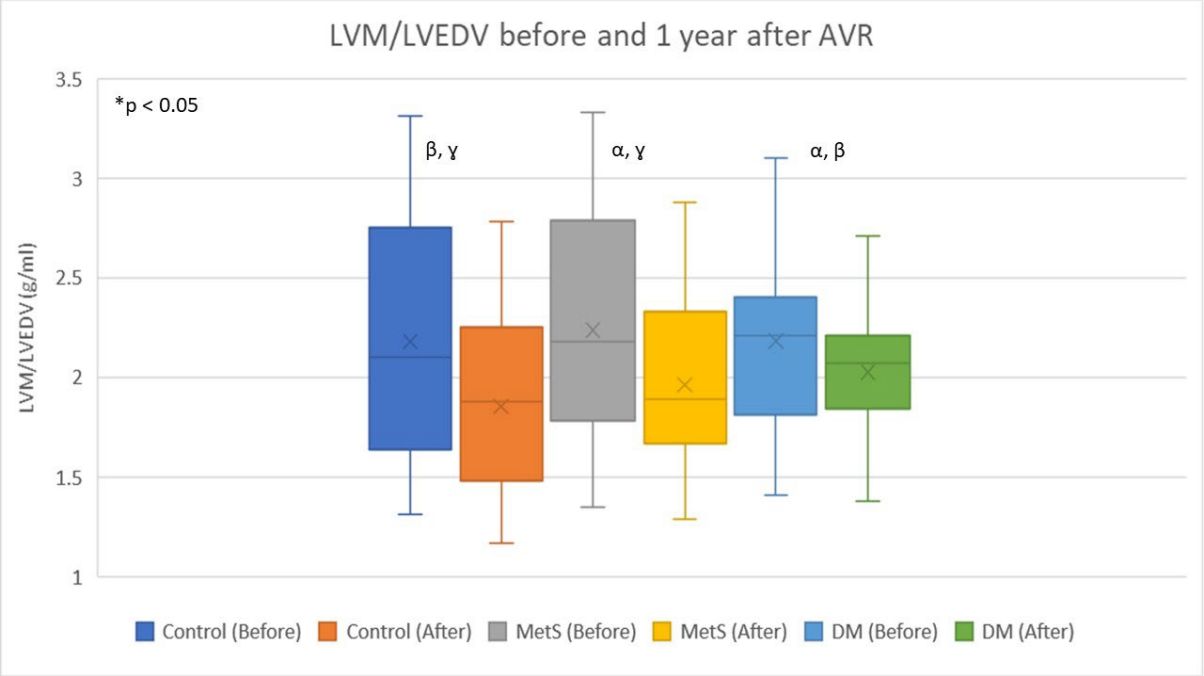


Figure 16. Box and whisker plot of the ratio of left ventricular mass to left ventricular end diastolic volume for patients before and after AVR. Boxes are presented with middle line (median), mean (cross), 25th percentile (lower box edge) and 75th percentile (upper box edge), Whiskers represent upper and lower adjacent values. P value represents results following 2-way ANOVA. LVM; left ventricular mass, LVEDV; left ventricular end diastolic volume, AVR; aortic valve replacement, MetS; metabolic syndrome, T2DM; type 2 diabetes mellitus. α = $p < 0.05$ compared with control; β = $p < 0.05$ compared with MetS; ɣ = $p < 0.05$ compared with T2DM

3.4 Discussion

The main findings of this study with respect to patients with severe AS undergoing isolated AVR are as follows:

(1) AS Patients without MetS or T2DM presented with both higher total cholesterol, LDL and cholesterol/HDL ratio;

(2) T2DM patients had increased weight, BMI and BSA at the time of surgery;

(3) Despite equivalence in conventional echocardiographic measurements confirming severe AS (i.e. peak and mean transvalvular gradients and aortic valve area), AS patients with MetS or T2DM demonstrated similarly higher LV mass and greater prevalence of LV hypertrophy which differed significantly from AS patients without MetS or T2DM;

(4) Following AVR, AS patients with MetS benefitted from improved left ventricular reverse remodelling as measured by a reduction in LVM/LVEDV ratio (but not a reduction in left ventricular mass alone) compared to patients with T2DM, although this improvement was not as marked as evidenced in patients without MetS or T2DM;

(5) All three groups benefitted from left ventricular mass regression following AVR.

These results allude to a difference in the aetiology of AS in patients with MetS and/or T2DM compared to patients without MetS or T2DM, as the latter had significantly elevated lipid parameters. Although the correlation between lipid profile and arteriosclerotic disease is well established, this study aimed to negate (or at the very least minimize) this confounding factor by omitting patients with concurrent coronary artery disease (the effect of extracardiac arteriopathy was also not significant in this study) which differs from the study by Guzzetti et al. It is likely that the prevalence of metabolic syndrome was likely underestimated in this study due to the unavailability of waist circumference measurements in the dataset. One potential explanation for the increase in LV mass and volume (and subsequently poorer regression of these following AVR) in

MetS and T2DM patients is an active subclinical inflammatory and oxidative state seen in patients with insulin resistance which is responsible for ventricular and aortic stiffening which acts synergistically with stenosis of the aortic valve. This would explain the finding of proportionally more MetS and T2DM patients with concentric and eccentric hypertrophy prior to AVR and the less pronounced improvement in left ventricular geometry (and mass regression) following AVR, as a degree of inherent stiffening of the myocardium is already present. The seemingly contradictory findings of significant improvement in mass-to-volume ratio but not absolute mass between the MetS and T2DM groups hints at histological changes of the myocardium causing a change in density. Extracellular expansion and cardiac fibrosis are both recognised consequences of hypertension and insulin resistance; both of which form part of the criteria defining metabolic syndrome. An overarching view of this study adds to the current viewpoint that MetS is part of a continuum culminating in T2DM as an endpoint, rather than two distinct pathologies. Given the differences in ventricular mass and dimensional profile for similarly severe AS, it is fair to conclude that ventricular remodelling occurs subclinically in the absence of symptoms and before the development of high transvalvular gradients and reduced aortic valve area. As all three groups in this study demonstrated favourable reverse-remodelling profiles following AVR, consideration could be given to offering AVR as a therapeutic option for promoting this beneficial phenomenon even in patients with mild or moderate AS, as LV remodelling and hypertrophy has been implicated in increased mortality in these patients.

3.5 Conclusion

MetS patients with severe AS present with a similar ventricular remodelling profile as the AS patients with T2DM and these remodelling profiles are in turn significantly different from those in the AS patients without MetS or T2DM. Aortic valve replacement surgery leads to improved reverse remodelling in metabolic syndrome patients compared to AS patients with T2DM. There may be a

role for aortic valve replacement in patients without severe, symptomatic AS as a therapeutic modality which promotes reverse-remodelling in selected patients in the future if the chances of achieving reverse-remodelling can be reliably predicted.

Chapter 4. Candidate Markers for Prediction of Left Ventricular Remodelling in Severe Aortic Stenosis Patients with Type 2 Diabetes or the Metabolic Syndrome

4.1 Introduction

Cardiac remodelling refers to any combination of molecular, cellular and interstitial changes in the myocardium causing deviation from normal anatomy (in terms of geometry, size and mass) and physiology (volume and pressure changes) of the heart, therefore ultimately culminating in deterioration of heart function and clinical status of the patient (54). The aetiologies underpinning this phenomenon and the current evidence base have been reviewed in Chapter 1. Briefly, left ventricular remodelling may occur as a compensatory mechanism aiming to preserve adequate cardiac output. In aortic stenosis, the stimulus for this is the impedance of ejection of blood from the left ventricular outflow tract across the stenosed (narrowed) aortic valve into the aortic root (55). In hypertension, the cumulative effect of elevated systemic vascular resistance from the entire arterial tree results in increased back-pressure which the contracting left ventricle has to overcome in ejection (56). The proposed aetiology is slightly different in coronary artery disease, where suboptimal myocardial oxygenation secondary to impaired blood flow in the diseased (atherosclerotic) coronary arteries reduces the force of ventricular contraction in systole, resulting in an increase in end systolic volume (the volume of blood retained) (57-60).

Left ventricular remodelling in diabetes is unique, as these structural and functional changes occur in the absence of the milieu of chronic pressure overload or the potential ischaemic effect of overt coronary artery disease. Evidence for several mechanisms, notably inflammation, impaired glucose tolerance and increased intramyocardial lipotoxicity, have been described (111). In the clinical

environment, it is difficult to meaningfully distinguish between mechanisms of diabetic remodelling from those of aortic stenosis, chronic hypertension and coronary artery disease as most (if not all) of these conditions can manifest in the same individual. The effect of this crossover of processes may be synergistic in both the onset and propagation of remodelling changes. The knowledge of factors associated with remodelling is therefore most useful initially in the assessment of disease severity, and potentially in risk prediction in asymptomatic patients.

The utility of 2-Dimensional transthoracic echocardiography (TTE) in both the assessment of valve and myocardial function and geometry is well established and is both sensitive and specific to geometrical changes heralding ventricular remodelling. There is good association with these parameters (validated by various magnetic imaging resonance studies) in the assessment of the severity of remodelling changes, including ascertaining pathologies of true myocardial hypertrophy, extracellular expansion and myocardial fibrosis. TTE can therefore be used to assess the association and correlation of candidate serum biomarkers with evidence of LV remodelling.

4.2 Materials and Methods

An observational study was proposed, and ethical approval was granted by the Hampshire B National Research Ethics Committee (NRES) – South Central (REC Ref: 18/SC/0162). University Hospital Southampton NHS Foundation Trust Research and Development department is acting as the local sponsor (IRAS ID: 240397, Ref: RHM CAR 0541). Patients between the ages of 40 and 90 years with severe aortic stenosis who were referred for first-time aortic valve replacement surgery at this institution were invited and consented to participate.

Exclusion criteria were the following:

1. Age < 40 years or > 90 years
2. Previous or active infective endocarditis
3. Redo Aortic Valve Surgery

4. Active Cancer

5. Inflammatory conditions which may affect the results of blood biomarkers

6. Patients on renal replacement therapy

7. Emergency surgery – as decided by the Consultant Surgeon.

In total 42 patients were recruited and following assessment of demographic and clinical parameters, were subdivided into three categories; AS patients without T2DM or MetS (Control, n=20), AS patients with MetS (n=9) and patients with confirmed T2DM (n=13).

4.2.1 Preoperative demographic and clinical data

Demographic and clinical data were prospectively collected from entries in both paper and electronic institutional records. The following categorical and continuous variables were ascertained; age, gender, height, weight, body mass index (BMI), body surface area (BSA), New York Heart Association Functional Classification for breathlessness (NYHA), Canadian Cardiovascular Society grading of angina pectoris (CCS), European System for Cardiac Operative Risk Evaluation (EuroSCORE), logistic EuroSCORE, hypertension (blood pressure ≥ 140/90 mmHg or on antihypertensive medication), extracardiac arteriopathy (carotid, aortoiliac, abdominal or peripheral arterial disease) and smoking status. Biochemical data included serum cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), glycated haemoglobin A1C (HbA1c) and fasting serum glucose. Patients were deemed positive for MetS if they exhibited any three of the following criteria proposed by the National Cholesterol Education Program - Adult Treatment Panel III (1) blood pressure ≥ 130/85 mmHg or receiving drug therapy for hypertension (2) fasting serum glucose ≥ 5.6 mmol/L (3) serum triglyceride ≥ 1.695 mmol/L (4) serum HDL < 1.04 mmol/L in men and < 1.30 mmol/L in women.

4.2.2 Echocardiography studies

As per the research protocol, I undertook comprehensive two-dimensional echocardiography prior to AVR and performed the subsequent calculations and measurements. All studies were performed using an iE33 ultrasound platform equipped with aS3 sector array probe (Philips Medical Systems, Best, The Netherlands) and the following parameters were measured or calculated; ejection fraction (EF), stroke volume index, peak systolic gradient across the aortic valve, mean systolic gradient across the aortic valve, aortic valve area (and subsequent indices by height and BSA), left atrial diameter, left ventricular end systolic diameter (LVESD), left ventricular end diastolic diameter (LVEDD), interventricular septum diameter (IVS), posterior wall thickness (PWT), left ventricular mass (subsequently indexed for height), incidence of LV hypertrophy and relative wall thickness ratio. Stroke volume was calculated by multiplying the flow velocity-time integral by the LV outflow tract area. Left ventricular mass was calculated using the modified cube formula and indexed to body surface area and to a 2.7 power of height. Left ventricular hypertrophy was defined as an indexed LV mass > 49 $g/m^{2.7}$ in men and > 47 $g/m^{2.7}$ in women. Relative wall thickness ratio was calculated as the ratio of 2 times the posterior wall thickness to LV internal diameter in diastole.

4.2.3 Serum acquisition

Additional peripheral blood sampling was undertaken for adiponectin, leptin, resistin, apolipoprotein A1, apolipoprotein B, lipoprotein A. These were performed on the day of surgery following induction of anaesthesia and insertion of a radial arterial monitoring line (as a routine part of cardiac surgery). 10ml of blood was initially drawn and discarded (to prime the arterial catheter) and a further 12ml of blood collected at normal body temperature into cryovials (all patients had been fasting for a minimum of 6 hours). These samples were transferred on ice to the laboratory and spun into serum in accordance with the extraction protocol describes in Chapter 2. These samples were then labelled, frozen at -70°C for storage while the appropriate number of patients were recruited. The samples were then sent for analysis (Cambridge Biomedical Assay Laboratories, Addenbrookes, UK).

4.2.4 Statistical Analysis

Data were analysed using both Microsoft Excel (version 1908; Microsoft, USA) and SPSS software (version 26; IBM, USA). Data were tested for normal distribution using the Shapiro-Wilk test. Continuous data were presented as mean ± SD, median was compared using paired and unpaired Student's t-test, ANOVA, or a Wilcoxon rank sum test. Categorical data were expressed as a percentage and compared using Fisher's test. Paired statistical tests were used to compare changes before and after aortic valve replacement. Post-hoc analysis for multiple groups was done using either the Holm-Sidak or Kruskal-Wallis tests as appropriate.

4.3 Results

Baseline clinical data are listed in Table 6. After accounting for exclusions, 42 AS patients were divided into three groups for analysis: AS patients without MetS or T2DM (Control) = 20, 47.6%; AS patients with MetS = 9, 21.4%; and AS patients with confirmed T2DM = 13, 31%. Between these groups, no significant differences in age ($p = 0.24$), height ($p = 0.96$), weight ($p = 0.76$) or gender ($p = 0.83$) was observed. The control group demonstrated a significant difference in mean body mass index (BMI = 23.5 ± 2.52 kg m^{-2}, $p < 0.001$) and mean body surface area (BSA = 1.89 ± 0.32 m^2, $p = 0.039$). No significant differences were seen in breathlessness or angina status (by NYHA and CCS Class), EuroSCORE, hypertension, smoking status, extracardiac arteriopathy or serum creatinine levels.

Assessment of routine lipid profile only demonstrated a significant difference in triglyceride levels between all three groups ($p = 0.001$), but not in total cholesterol, HDL, LDL or cholesterol to HDL ratio. As expected, both fasting serum glucose and glycated haemoglobin (HbA1C) showed significant differences between all three groups ($p < 0.001$ in both cases).

Table 6. Baseline demographic and laboratory data

Variable (N = 42)	Control n=20 (47.6%)	MetS n=9 (21.4%)	T2DM n=13 (31%)	p value
Age, years	71 ± 3.74	69 ± 4.75	70 ± 4.25	0.24
Height, m	1.68 ± 0.14	1.70 ± 0.34	1.69 ± 0.33	0.96
Weight, kg	79.2 ± 3.2	80.5 ± 6.95	79.5 ± 3.29	0.76
Male gender, n	10 (50%)	6 (66.67%)	8 (61.5%)	0.83
BMI, kg m^{-2}	23.5 ± 2.52	28.5 ± 2.01	27.1 ± 2.02	**< 0.001**
BSA, m^2	1.89 ± 0.32	2.23 ± 0.54	2.28 ± 0.57	**0.039**
NYHA				
Class I	10 (50%)	3 (33.4%)	6 (46.2%)	
Class II	8 (40%)	5 (62.5%)	6 (46.2%)	
Class III	2 (10%)	1(11.1%)	1 (7.6%)	
Class IV	0 (0%)	0 (0%)	0 (0%)	
CCS				
Class I	12 (60%)	4 (44.5%)	5 (38.5%)	
Class II	6 (30%)	5 (55.5%)	6 (46.1%)	
Class III	2 (10%)	0 (0%)	2 (15.4%)	
Class IV	0 (0%)	0 (0%)	0 (0%)	
EuroSCORE	6.05 ± 0.37	6.55 ± 2.87	7.12 ± 0.62	0.1
Hypertension	11 (55%)	6 (66.7%)	9 (69.2%)	0.86
Current/Ex-smoker	11 (55%)	4 (44.5)	3 (23.1%)	0.39
Extracardiac Arteriopathy	1 (5%)	0 (0%)	2 (15.4%)	0.37
Creatinine, µmol/L	85 ± 17.6	86 ± 15.1	92.3 ± 20	0.53
Total Cholesterol, mmol/L	4.66 ± 1.74	4.64 ± 1.26	4.52 ± 0.81	0.96
Triglycerides, mmol/L	1.15 ± 0.55	1.48 ± 0.35	1.86 ± 0.82	**0.001**
HDL, mmol/L	1.44 ± 0.48	1.44 ± 0.4	1.34 ± 0.43	0.79
LDL, mmol/L	3.29 ± 1.6	3.21 ± 1.13	3.18 ± 1.01	0.97
Cholesterol/HDL ratio	3.52 ± 1.54	3.34 ± 0.85	3.25 ± 1.07	0.83
Fasting blood glucose, mmol/L	5.3 ± 1.11	6.0 ± 0.99	8.8 ± 1.19	**< 0.001**
HbA1C, mmol/mol	32 ± 8.8	41 ± 4.1	54 ± 6	**< 0.001**

Data presented as mean ± SD or n (%). P-values obtained from one-way ANOVA and post-hoc Holm-Sidak or Kruskal-Wallis correction as appropriate (α = $p < 0.05$ compared with control; β = $p < 0.05$ compared with MetS; ɣ = $p < 0.05$ compared with T2DM). BMI, body mass index; BSA, body surface area; NYHA, New York Heart Association functional class of breathlessness; CCS, Canadian Cardiovascular Society grading of angina pectoris; EuroSCORE, European System for Cardiac Operating Risk Evaluation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1C, glycated haemoglobin A1C.*Hypertension = blood pressure ≥140/90 mmHg or on antihypertensive medication; extracardiac arteriopathy = carotid, aortoiliac, abdominal or peripheral arterial disease.

Table 7. Candidate markers for inflammation, calcification and metabolic activity

Variable (N = 42)	Control n=20 (47.6%)	MetS n=9 (21.4%)	T2DM n=13 (31%)	p value
C-reactive protein (CRP), nmol/L	60 ± 25	52 ± 31	90 ± 11	0.36
Platelets x 10^9/L	251 ± 114	348 ± 86	327 ± 51	**0.017**
Neutrophil count x 10^9/L	4.19 ± 1.38	4.16 ± 1.19	4.19 ± 13.8	0.39
Lymphocyte count x 10^9/L	1.78 ± 0.61	1.74 ± 0.72	2.14 ± 0.73	0.26
Neutrophil to lymphocyte ratio (NLR)	2.60 ± 1.33	2.7 ± 0.96	2.61 ± 1.66	0.98
Platelet to lymphocyte ratio (PLR)	164 ± 95.7	256 ± 210	174 ± 80	0.18
Adiponectin, µg/ml	9.48 ± 4.52	5.94 ± 1.07	5.19 ± 1.75	**0.002**
Leptin, ng/ml	26.0 ± 19.5	15.7 ± 10.8	14.4 ± 7.44	*0.068*
Resistin, pg/ml	9617.7 ± 2424.9	11975 ± 4022	15393 ± 8060	**0.011**
Apolipoprotein A1 (Apo A1), g/ml	0.61 ± 0.13	0.70 ± 0.15	0.69 ± 0.13	*0.08*
Apolipoprotein B (Apo B), g/ml	0.26 ± 0.11	0.40 ± 0.17	0.38 ± 0.14	**0.011**
Lipoprotein A (Lp(a)), g/ml	0.19 ± 0.14	0.38 ± 0.25	0.37 ± 0.23	**0.012**

Data presented as mean ± SD or n (%). CRP, C-reactive protein; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; Apo A1, Apolipoprotein A1; Apo B, Apolipoprotein B; Lp(a), Lipoprotein A. *p-values obtained from one-way ANOVA and post-hoc Holm-Sidak correction $\alpha = p < 0.05$ compared with control; $\beta = p < 0.05$ compared with MetS; $\gamma = p < 0.05$ compared with T2DM.

Table 7 presents the candidate biomarkers for LV remodelling. These include the adipokines and lipoproteins described in the Materials and Methods section, along with other clinical parameters taken at the time of surgery. Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) are synthesized markers that have been shown to correlate with subclinical inflammation in various disease processes. Their significance in both AS and T2DM has not been established, hence warrants attention in this study.

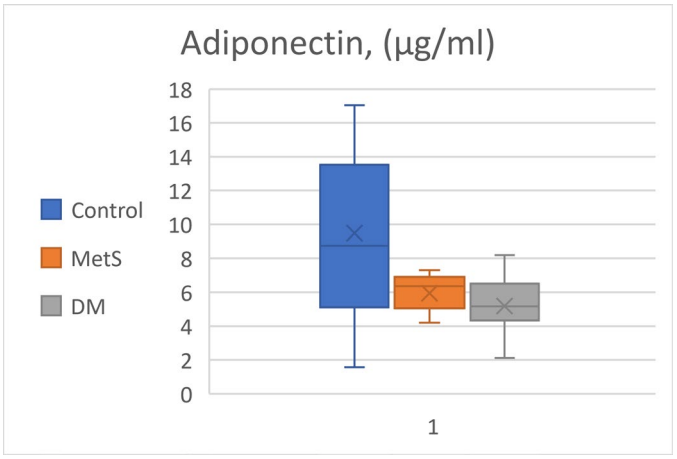
Significant differences between the Control group and the MetS/T2DM groups were with respect to platelet count ($p = 0.017$), adiponectin ($p = 0.002$), apolipoprotein B ($p = 0.011$) and lipoprotein A ($p = 0.012$) levels. Between the Control and Mets/T2DM cohorts, it is noteworthy to mention that leptin and apolipoprotein A1 differences are approaching significance (leptin, $p = 0.068$ and ApoA1, $p = 0.08$). Resistin, however, was significantly different between the T2DM and Control/MetS cohort ($p = 0.011$). No significant differences were observed for measurements of C-reactive protein, neutrophil count, lymphocyte count, NLR or PLR within or between groups.

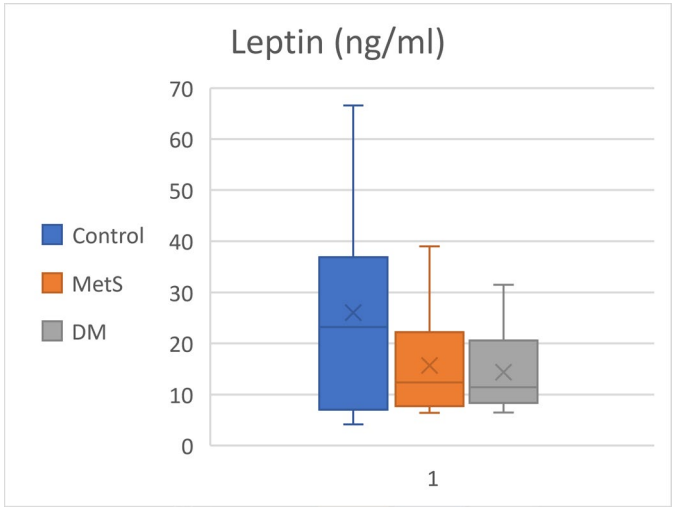
Table 8. Two-dimensional transthoracic echocardiography measurements

Variable N = 42	Control n=20 (47.6%)	MetS n=9 (21.4%)	T2DM n=13 (31%)	p value
Ejection fraction, %	63.6 ± 6.24	64.1 ± 12.75	64.3 ± 13.5	0.99
Stroke volume index, ml/$m^{2.04}$	29 ± 4.5	26 ± 4.14	26 ± 6.18	0.1
Peak gradient, mmHg	64 ± 19.4	62 ± 12.9	64 ± 11.2	0.97
Mean gradient, mmHg	50 ± 7.46	52 ± 7.93	53 ± 5.34	0.45
Aortic Valve Area, cm^2	0.77 ± 0.13	0.75 ± 0.12	0.60 ± 0.19	**< 0.01**
AVA indexed by BSA, cm^2/m^2	0.8 ± 0.16	0.75 ± 0.14	0.61 ± 0.25	**0.025**
AVA indexed by height, $cm^2/m^{2.04}$	0.52 ± 0.21	0.50 ± 0.14	0.44 ± 0.26	**0.043**
Left Atrial Diameter, cm	3.4 ± 0.7	4.0 ± 0.49	5.6 ± 0.88	**< 0.001**
LVESD, cm	2.83 ± 0.36	2.89 ± 0.38	2.65 ± 0.30	0.21
LVEDD, cm	5.05 ± 0.98	5.14 ± 0.20	5.31 ± 1.33	0.79
IVS, cm	1.25 ± 0.33	1.35 ± 0.24	1.42 ± 0.34	0.39
PWT, cm	1.14 ± 0.18	1.21 ± 0.38	1.22 ± 0.23	0.63
LV mass indexed by height, $g/m^{2.7}$	54.1 ± 10.1	59.5 ± 12.75	61.2 ± 0.58	**0.028**
LV Hypertrophy	8 (40%)	7 (77.8%)	8 (61.5%)	**0.041**
Relative wall thickness ratio	0.49 ± 0.12	0.59 ± 0.11	0.58 ± 0.08	**0.033**

Data presented as mean ± SD or n (%). P-values obtained from one-way ANOVA and post-hoc Holm-Sidak or Kruskal-Wallis correction as appropriate ($\alpha = p < 0.05$ compared with control; $\beta = p < 0.05$ compared with MetS; $\gamma = p < 0.05$ compared with T2DM). AVA, aortic valve area; BSA, body surface area; LVESD, left ventricular end systolic diameter; LVEDD, left ventricular end diastolic diameter; IVS, interventricular septum diameter; PWT, posterior wall thickness; RWT, relative wall thickness; LV, left ventricle; LVM, left ventricular mass

Table 8 summarizes differences in preoperative 2-dimensional transthoracic echocardiographic (TTE) measurements in these patients prior to undergoing isolated aortic valve replacement for AS. Between the three groups, no significant difference in ejection fraction ($p = 0.99$), stroke volume index ($p = 0.1$), peak gradient ($p = 0.97$) and mean gradient ($p = 0.45$) were observed. AS patients with T2DM demonstrated significantly reduced aortic valve area (0.60 ± 0.19 cm^2, $p < 0.01$), aortic valve area indexed to body surface area (0.64 ± 0.26 cm^2/m^2, $p = 0.025$) and for aortic valve area indexed by height (0.44 ± 0.26 $cm^2/m^{2.04}$, $p = 0.043$). In terms of dimensional variation, AS patients with T2DM had larger left atrial diameters (5.6 ± 0.88cm, $p < 0.001$) compared to the Control/MetS groups. On the other hand, the control group demonstrated reduced left ventricular mass (indexed by height; 54.1 ± 10.1 $g/m^{2.7}$, $p = 0.028$) and relative wall thickness ratio (RWT; 0.49 ± 0.12, $p = 0.033$). No significant differences were observed within or between groups for left ventricular end systolic diameter (LVESD), left ventricular end diastolic diameter (LVEDD), interventricular septal diameter (IVS), posterior wall thickness (PWT), or LV hypertrophy.





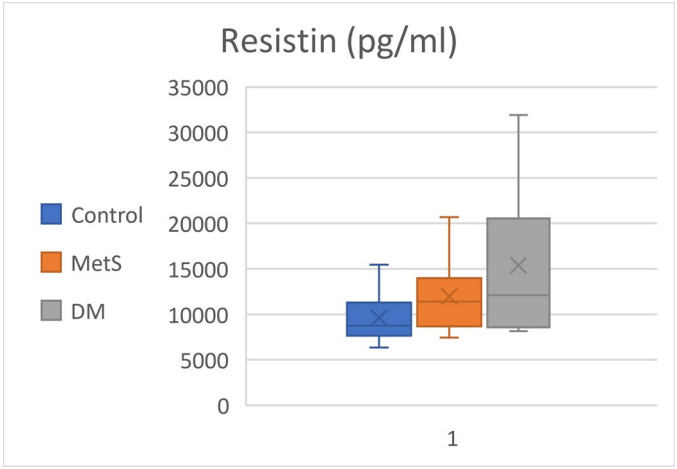
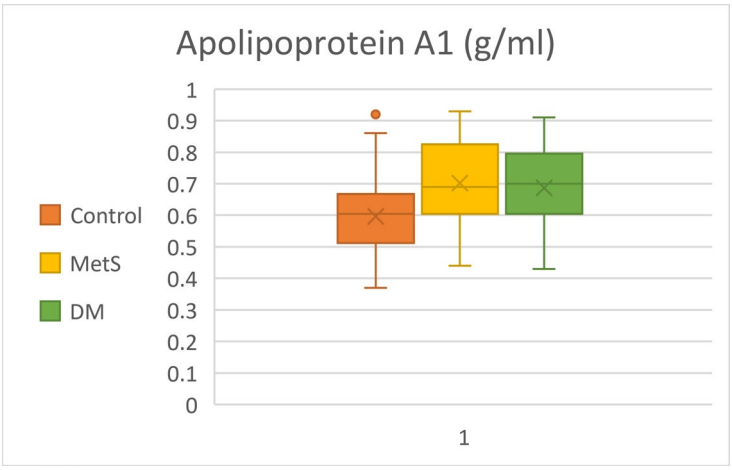
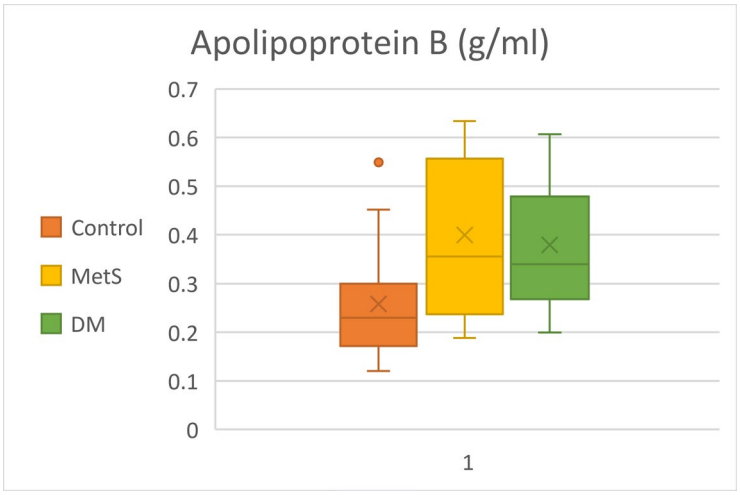


Figure 17. Box and whisker plot describing the difference in adipokine levels (adiponectin, leptin and resistin) in AS patients without MetS or T2DM (Control), AS patients with metabolic syndrome (MetS) and patients with type 2 diabetes (T2DM). Boxes are presented with median (middle line), mean (cross), 25th percentile (lower box edge) and 75th percentile (upper box edge), Whiskers represent upper and lower adjacent values. P value represents results following 2-way ANOVA. α = $p < 0.05$ compared with control; β = $p < 0.05$ compared with MetS; ɣ = $p < 0.05$ compared with T2DM





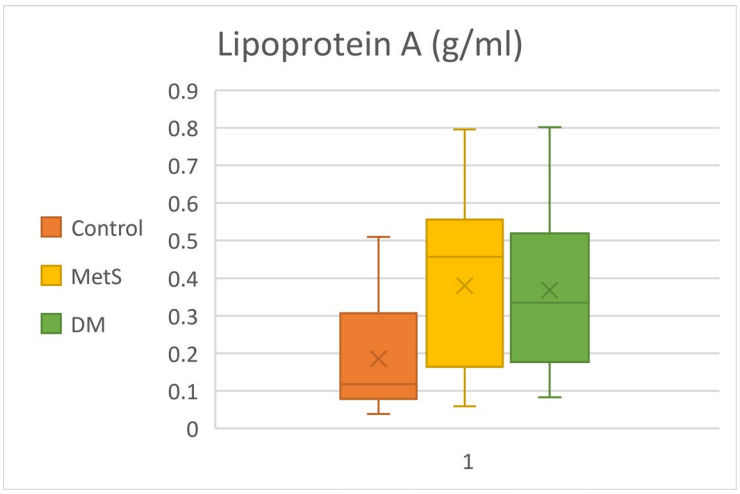


Figure 18. Box and whisker plot describing the difference in apolipoprotein levels (apolipoprotein A1, apolipoprotein B and Lipoprotein A) in patients without metabolic syndrome or type 2 diabetes (Control), patients with metabolic syndrome (MetS) and patients with type 2 diabetes (T2DM). Boxes are presented with median (middle line), mean (cross), 25th percentile (lower box edge) and 75th percentile (upper box edge), Whiskers represent upper and lower adjacent values. P-value represents the results following 2-way ANOVA. $\alpha = p < 0.05$ compared with control; $\beta = p < 0.05$ compared with MetS; $\gamma = p < 0.05$ compared with T2DM

4.4 Discussion

4.4.1 Adipokines and subclinical left ventricular remodelling

Analysis of all these particular candidate markers either demonstrated significant variation (or are approaching significance) in a cohort with severe AS. Recent interest in adipokines in relation to cardiovascular disease has focused on arterial disease and its potential underlying mechanisms, with a consensus in the literature that adiponectin exhibits cardioprotective properties, and leptin and resistin as mediators of inflammation and oxidative stress and vascular smooth muscle hypertrophy (although the evidence for resistin is limited and conflicting) (112-115). Given that our study cohort was free of coronary artery disease (all patients underwent coronary angiography to confirm disease-free status), we are better able to evaluate the association of these adipokines without the confounding factor of subclinical ischaemic cardiac disease being a cause of left ventricular remodelling. Thus, only two potential aetiological mechanisms (in the absence of cardiac amyloid disease – this is beyond the scope of this thesis, and not evidenced in this cohort) can be scrutinised – chronic pressure overload (from AS) and the inflammatory state linked to impaired glucose tolerance (from MetS and T2DM) (116, 117). All patients met the criteria for severe AS with or without symptoms, and analysis of peak and mean transvalvular gradients were equivocal in all groups. The only significant difference in the parameters that form the criteria for severe AS was the aortic valve area (both native and indexed) – this was reduced in patients with T2DM as compared to the Control and MetS groups. Such a finding would indicate that patients with T2DM have a heavier "stenotic burden" of the aortic valve for similar left ventricular function and aortic valve function than their non-diabetic counterparts. Given that changes in ventricular volume throughout the cardiac cycle were similar in all groups (as evidenced by the absence of significant differences in the dynamic parameters LVESD and LVEDD), two possible explanations are: 1) that the same volume of non-ejected blood in the ventricle exerts different pressure effects on the ventricular walls – this can either be the cause, or consequence, of myocardial stiffening, and 2) the volume of non-ejected

blood is greater, but this is not observed via the parameters of ventricular geometry (LVESD and LVEDD) as this excess blood is decanted elsewhere – in this case, the left atrium. Indeed, the diabetes cohort had larger left atriums (as evidenced by the significant increase in LA diameter). When this model is applied in consideration of the MetS cohort, it is noted that there is significant left atrial enlargement compared to the control group despite the similarity in aortic valve area. In this case, this model would suggest that maladaptation of the aortic valve could be responsible for causing pressure changes ultimately leading to left atrial enlargement even when the reduction in valve area is not yet significant. We stress here that none of these patients had echocardiographic evidence of mitral valve regurgitation, hence backflow of blood from the left ventricle into the left atrium must have been occurring insidiously; in very small quantities over a long period of time.

4.4.2 Lipoproteins and potential myocardial lipotoxicity

The significance of lipoproteins in cardiovascular disease is well documented, with the mechanisms attributable to alterations in the lipid pathway (118). Despite mechanisms of lipid infiltration (and subsequent inflammation) causing calcification being well defined in arterial disease, the evidence for controlling lipid parameters in isolated aortic stenosis is not conclusive (119,120). A consistent trend was noted in all three lipoproteins showing significantly elevated levels in the MetS/T2DM groups compared to the Control group suggesting a similar lipid burden in the MetS/T2DM groups. At a glance, the conventional serum lipid panel of total cholesterol, HDL, LDL and cholesterol to HDL ratio shows no association with the lipoprotein profile with the exception of triglyceride levels. This alludes to recently suggested hypotheses that the serum lipid profile is a poor marker of organ-specific lipotoxicity – the theory that localised lipid-stimulated or propagated activity of microvascular inflammation, oxidation and vascular smooth-muscle hypertrophy occurs in various visceral fat depots enveloping different organs (i.e. heart, liver, pancreas, and bowel).

Chapter 5. General Discussion

5.1 Introduction

This thesis was born from the perspective of a clinician dealing with patients with severe aortic stenosis – the tail end of a progressive pathological process which evades scrutiny for years due to its asymptomatic nature in the early stages. Even if milder forms of aortic stenosis were detected by chance, there is currently neither any lifestyle nor dietary modification or pharmacological therapies that can meaningfully retard the progression towards severe aortic stenosis which may result in myocardial infarction, syncope or even sudden death. As outlined in Chapter 1 if surgical or transcatheter aortic valve replacement is not (or cannot) be performed, cardiac failure inevitably occurs leading to death. Patients with Type 2 Diabetes Mellitus and the constellation of conditions that make up the Metabolic Syndrome make up a significant proportion of our patients, hence efforts to better understand why these patients are more adversely affected both by severe aortic stenosis will ultimately improve our institutional practice, to the benefit of the Wessex population undergoing cardiac surgery.

The steady increase in the global burden of cardiovascular disease and associated sequelae is due to, in no small part, the initial prolonged asymptomatic subclinical progression of processes at a cellular level. Current clinical practice utilises parameters of disease status - rather than precursory harbingers of the disease process itself – to inform decisions regarding the necessity and urgency of medical intervention. Indeed, hypertension is still widely regarded as a marker of increased risks of secondary cardiovascular events (such as myocardial infarction, heart failure and stroke), rather than being recognised as an established pathological entity secondary to genetics or activation of neurohormonal systems (such as the sympathetic nervous system and renin-angiotensin-aldosterone system). The last two decades have brought about great advancements in the

understanding of the role of different lipoprotein densities and triglycerides (i.e. the "lipid profile") which led to the subsequent development of targeted treatment with HMG-CoA reductase inhibitors (statins). The overwhelming success of statin therapy in reducing the risk of secondary cardio- and cerebrovascular events is well established; however, the current aetiological gap between genetic predisposition and endothelial damage leading to the chain of events resulting in the formation of atherosclerotic plaque needs to be addressed, as this would form the basis of truly preventative medical intervention. Until then, the identification of markers of disease progress at a stage when deleterious effects can be minimized remains a clinically valuable metric. The fact that several studies (discussed previously) have failed to demonstrate the beneficial effects of statin therapy in aortic stenosis compared to its efficacy in coronary artery disease points to a difference in mechanisms of calcification in these two pathologies.

The phrase "Let not the perfect be the enemy of the good" may succinctly outline the reasoning behind the widespread use of the term "Metabolic Syndrome" – a realisation that this author concurs with following the concluding work of this manuscript. Connections between the pathological processes resulting from hypertension, hypertriglyceridemia, obesity and raised plasma glucose are nebulous at best. This however does not diminish the practical utility of the term "Metabolic Syndrome" in identifying patients at a higher risk of cardiovascular sequelae and should be stratified as such. Although research efforts should continue to chip away at uncovering causal factors – important as these can then form the basis of targeted therapies – associative factors are equally valuable, especially in the patient-facing environment of clinical practice.

5.2 Impact of the COVID-19 Pandemic on the study

Like many other research studies at institutions around the globe, the COVID-19 coronavirus pandemic had an impact on the original design of this study, necessitating modifications. Participants were not able to attend for follow-up serum biomarker sample acquisition and follow-up transthoracic echocardiography due to an institution-wide charter to prioritize only essential COVID-19 related research trials at the time. Such follow-up data would provide valuable insights. Nevertheless, the analysis of data collected yielded interesting findings that can inform clinical practice – An overview of these findings and insights garnered from these will be discussed further.

5.3 Overview of Study and Key Findings

This study incorporated two separate cohorts, the first of which was with a view to identifying differences in the manifestation of left ventricular remodelling (and subsequent reverse-remodelling) in aortic stenosis patients with and without Metabolic Syndrome and Type 2 Diabetes Mellitus. Data were collected and analysed from 367 patients with isolated aortic valve stenosis who attended follow-up echocardiography. The second dataset focused on the correlation between serum adipokines and lipoprotein levels in a prospective set of patients (n = 42) undergoing isolated aortic valve replacement for severe aortic stenosis at the same institution.

5.3.1 Preoperative demographic data

The background demographic data of the patients revealed some differences with may account for differences in the metabolic profile of the control group and patients with MetS or T2DM. The control group had higher levels of total cholesterol, LDL and cholesterol to HDL ratios. The T2DM group has increased weight, body mass index and body surface area at the time of surgery. This could allude to differences in molecular pathways causing aortic stenosis in these groups and was in

part responsible for our selection of adipokines and lipoproteins as an observed variable in our next (prospective) patient cohort.

5.3.2 Differences in left ventricular geometry in patients with and without MetS/T2DM

Preoperatively, there were statistically significant differences in parameters of LV geometry between patients without MetS/T2DM, patients with MetS and patients with T2DM. Post-hoc analysis of two-way ANOVA with Holm-Sidak and Kruskal-Wallis corrections enabled comparisons of P-values between these three groups – this added an enlightening dimension for analysis, as it highlighted that the parameters of LV mass, LV mass indexed by height, LV hypertrophy, posterior wall thickness and relative wall thickness ratio all demonstrated significant differences between MetS/T2DM and the control group, but not between MetS and T2DM patients (note that these parameters suggested a higher degree of remodelling in both the MetS and T2DM groups compared to the control group). This finding confirmed that MetS and T2DM patients were equally susceptible to similar degrees of ventricular remodelling in severe AS. Analysis of follow-up echocardiography one year after surgery revealed significant improvements in left ventricular hypertrophy in all three groups, but no significant differences in improvement between groups (i.e. with regards to reduction of LV hypertrophy, all groups benefitted from the surgery, but no one group benefitted more than the other two).

Further dissection of this data aimed to stratify the remodelling profile of these patients (i.e. normal, concentric remodelling, concentric hypertrophy and eccentric hypertrophy) based on validated criteria. In all three groups, there was statistically significant reverse remodelling; however, it was noted that these differences benefitted participants with concentric remodelling and concentric hypertrophy more than eccentric hypertrophy. It is worth mentioning that any improvement or deterioration resulted in a shift by only one level.

5.3.3 Left Ventricular Mass and Mass-to-Volume Ratio

Analysis of preoperative and postoperative left ventricular mass were analysed in the same fashion, with 3-way comparisons between these groups. There was a statistically significant reduction of left ventricular mass within each group, though the reduction of mass in the control group was statistically more pronounced than in the MetS and T2DM groups (Figure 15). The left ventricular mass to left ventricular end diastolic volume ration (LVM/LVEDV) was then calculated and compared. This now demonstrated significant differences within the groups before and after surgery (as predicted), however there was no significant differences between all three groups. This seemingly contradictory finding of significant improvement in mass-to-volume ratio, but not absolute mass, hints at structural changes of the myocardium at a histological level resulting in a change in density. Likely culprits include extracellular expansion and myocardial fibrosis, which are prevalent in states of insulin resistance and hypertension.

5.3.4 Candidate markers for left ventricular remodelling

A cohort of 42 prospective patients was enrolled on this study, all of whom were undergoing aortic valve replacement surgery for echocardiographically confirmed severe aortic stenosis in the absence of coronary artery disease. Preoperative peripheral blood sampling was performed and selected serum adipokines (adiponectin, leptin and resistin) and lipoproteins (apolipoprotein A1, apolipoprotein B and lipoprotein A) were extracted and quantified as per Chapter 2. Once again, these patients were sub-stratified into patients without Metabolic Syndrome or Type 2 Diabetes Mellitus (the control group), patients with Metabolic Syndrome (MetS) and patients with Type 2 Diabetes Mellitus (T2DM). The significant difference in fasting serum glucose and glycated haemoglobin (HbA1C) was observed. In addition, there was also a significant difference in triglyceride levels in the T2DM group. With regards to candidate markers for inflammation, calcification and metabolic activity (Table 7), there were significant differences in platelet count

(higher in the MetS and T2DM groups versus the control group), adiponectin (higher in the control group), resistin (higher in the T2DM group), Apolipoprotein B (higher in the MetS and T2DM groups versus the control group) and lipoprotein A (higher in the MetS and T2DM groups versus the control group). Notable parameters that are approaching significance in this cohort are leptin (raised in the control group versus the MetS and T2DM groups, P = 0.068) and apolipoprotein A1 (lower in the control group versus the MetS and T2DM groups, P = 0.08).

Preoperative transthoracic echocardiography in this cohort demonstrated significant differences in aortic valve area parameters (significantly reduced in T2DM versus MetS and the control group), left atrial diameter (significant differences between all three groups, increasing in the following order: control → MetS → T2DM), indexed left ventricular mass (raised in MetS and T2DM versus the control group), LV hypertrophy (significant differences between all three groups, increasing in the following order: control → MetS → T2DM) and relative wall thickness ration (raised in MetS and T2DM groups versus the control group). Following these findings, regression analyses were carried out using these statistically significant echocardiographic parameters as the independent variable (the axis) against one of the candidate markers in each subgroup (control, MetS and T2DM). This step was repeated for each candidate marker. None of these regression analyses demonstrated significance (F-value of < 0.05), suggesting that no single marker (or combination of the studied markers) reliably correlated with the echocardiographic findings.

Despite these correlations being overwhelmingly unrepresentative, this study adds further weight to the multimodal theory of valvular calcification and myocardial remodelling in patients with MetS and T2DM.

5.4 Summary of new knowledge and value added to current literature.

This study adds to the current literature on subclinical differences in people with type 2 diabetes mellitus or metabolic syndrome undergoing aortic valve replacement with the following findings:

1. People with pre-existing T2DM or MetS have more severe features of left ventricular remodelling at the time of consideration of surgical intervention for aortic stenosis compared to people without T2DM or MetS.
2. At one year following AVR, people with MetS demonstrated significantly more (beneficial) left ventricular reverse-remodelling than patients with T2DM, however improvement in both these cohorts were not as pronounced as in people without T2DM or MetS.
3. People with T2DM and MetS had increased levels of resistin, lipoprotein-A and apolipoprotein-B1 compared to people without T2DM or MetS. People without T2DM or MetS, on the other hand, had increased levels of adiponectin and leptin compared to the other two groups.

5.5 Limitations and Future Directions

One limitation of this study was the duration and by association, the chronology of latent severity of underlying disease states (i.e. hypertension, hyperglycaemia, hyperlipidaemia, obesity etc) prior to the presentation of severe aortic stenosis. Although this study was performed in a high volume of cardiac surgical centre in the UK, people will only be referred for surgery once severe aortic stenosis is well-established. Therefore, the preoperative demographic data in both these studies represents merely a snapshot of a patient's metabolic profile prior to surgery. An ideal study design would be to employ screening echocardiography, lifestyle questionnaires and biochemical (blood) tests within the general population to facilitate the detection of AS, impaired glucose tolerance, hyperlipidaemia, hypertension and obesity in very early stages. These people can then be followed up over a long

period with serial serum and echocardiography measurements to potentially identify a metabolic "tipping point" where pathological processes demonstrate increased propagative activity.

As mentioned in the introduction to this section, the COVID-19 pandemic had an impact on follow-up data collection. 1-year postoperative serum samples and transthoracic echocardiography was not performed on the 42 patients in the prospective cohort. Analysis of parameters for LV hypertrophy, mass and LV mass to LV end diastolic volume ratio would have been compared with postoperative levels of the adipokines and lipoprotein tested (alongside other potential markers such as platelet count, neutrophil to lymphocyte ratio and CRP). This data would have been invaluable in identifying the relationship between these biomarkers and LV status and clarify the cause or affect relationship between serum biomarker levels and changes in LV geometry and haemodynamic performance.

Another limitation is the sample size calculations which were performed and presented in Chapter 2. The cross-sectional and observational nature of these studies add a large factor of variability. Nevertheless, it was judged to be appropriate to include this to serve as a guide to aid recruitment.

Perhaps the biggest limitation of this study was the lack of access to cardiac magnetic resonance imaging scanning. Contrast-enhanced MRI is a non-invasive, clinically useful technique for accurate quantification and localization of myocardial fibrotic burden. Late-gadolinium enhancement imaging can be used to identify the presence, pattern, and size of replacement or focal fibrosis, and has proven prognostic capacity. During the setup phase of this study, consideration was given to obtaining biopsies of myocardial tissue to perform histological assessment for fibrosis. However, the consensus was that isolated, small volume samples would not be representative of the overall fibrotic burden or extracellular expansion of myocardium. Replacement fibrosis does not follow a set pattern of propagation. The findings from these biopsies would be sporadic, and attempts at correlation with other parameters would not be clinically valuable. Identification of patterns and quantification of changes in myocardial structure by cardiac magnetic resonance imaging would add

a valuable dimension to this study, as patterns of reverse-remodelling would provide useful insights into underlying processes.

5.6 Concluding Remarks

Patients with Metabolic Syndrome and Type 2 Diabetes who experience severe aortic valve stenosis with preserved preoperative left ventricular function present in a more advanced state of subclinical remodelling of the left ventricle, which in turn is more recalcitrant to the beneficial phenomenon of reverse remodelling following surgical aortic valve replacement. This would suggest that aside from chronic pressure overload, additional maladaptive mechanisms are at play resulting in structural changes to the left ventricle in this subset of patients. Differences in adipokine and lipoprotein serum profiles in these patients may suggest potentially different aetiologies of calcification and fibrotic processes. However, no correlation between these markers in isolation or collectively can account for differences in preoperative parameters of left ventricular function and geometry. This raises the question of whether people in earlier stages of left ventricular remodelling would derive more benefit from aortic valve replacement surgery. A further thought experiment, which is shared by the wider research community and emerging literature, is the potential of aortic valve replacement surgery in cases where aortic stenosis is less than severe in patients with metabolic syndrome or type 2 diabetes mellitus to reap the prognostic benefits of reverse remodelling. The findings of this thesis, humbly presented here, serves to add weight to this consideration.

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Appendix

i) Confirmation of Health Research Authority (HRA) approval

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Mr Sunil Ohri
Hamtun House
9a Westrow Road, Banister Park
Southampton
SO15 2NA

Email: hra.approval@nhs.net
Research-permissions@wales.nhs.uk

22 May 2018

Dear Mr Ohri

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Predictors of progression of calcific stenosis in patients with Type 2 Diabetes to identify indications and optimal timing to intervention.**
IRAS project ID:	**240397**
REC reference:	**18/SC/0162**
Sponsor	**Research & Development Department (Division D)**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

How should I continue to work with participating NHS organisations in England and Wales?
You should now provide a copy of this letter to all participating NHS organisations in England and Wales*, as well as any documentation that has been updated as a result of the assessment.

*'In flight studies' which have already started an SSI (Site Specific Information) application for NHS organisations in Wales will continue to use this route. Until 10 June 2018, applications on either documentation will be accepted in Wales, but after this date all local information packs should be shared with NHS organisations in Wales using the Statement of Activities/Schedule of Events for non-commercial studies and template agreement/ Industry costing template for commercial studies.

This is a single site study sponsored by the site. The sponsor R&D office will confirm to you when the study can start following issue of HRA and HCRW Approval.

It is important that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details of the research management function for each organisation can be accessed here.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within the devolved administrations of Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) has been sent to the coordinating centre of each participating nation. You should work with the relevant national coordinating functions to ensure any nation specific checks are complete, and with each site so that they are able to give management permission for the study to begin.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?
The document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

I am a participating NHS organisation in England or Wales. What should I do once I receive this letter?
You should work with the applicant and sponsor to complete any outstanding arrangements so you are able to confirm capacity and capability in line with the information provided in this letter.

The sponsor contact for this application is as follows:

Name: Claire Ayling
Tel: 02380777222
Email: DivisionDR7D@uhs.nhs.uk

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **240397**. Please quote this on all correspondence.

Yours sincerely

Chris Kitchen

IRAS project ID	240397

Assessor

Email: hra.approval@nhs.net

Copy to: *Ms Claire Ayling, University Hospital Southampton NHS Foundation Trust (Sponsor and R&D Contact)*

IRAS project ID	240397

List of Documents

The final document set assessed and approved by HRA and HCRW Approval is listed below.

Document	*Version*	*Date*
GP/consultant information sheets or letters [PROCAS - GP Letter v2.0 Highlighted changes]	2.0	24 April 2018
GP/consultant information sheets or letters [PROCAS - GP Letter v2.0]	2.0	24 April 2018
IRAS Application Form [IRAS_Form_12032018]		12 March 2018
Other [REC response - cover letter]		23 April 2018
Other [HRA response 23-04-2018]		23 April 2018
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Summary CV for Chief Investigator (CI) [CV - Sunil Ohri]	v1.0	15 March 2018
Summary CV for student [Summary CV - Suresh]	v1.0	15 March 2018
Summary CV for supervisor (student research) [CV - Sunil Ohri]	v1.0	15 March 2018

IRAS project ID	240397

Summary of assessment

The following information provides assurance to you, the sponsor and the NHS in England and Wales that the study, as assessed for HRA and HCRW Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England and Wales to assist in assessing, arranging and confirming capacity and capability.

Assessment criteria

Section	Assessment Criteria	Compliant with Standards	Comments
1.1	IRAS application completed correctly	Yes	No comments
2.1	Participant information/consent documents and consent process	Yes	No comments
3.1	Protocol assessment	Yes	No comments
4.1	Allocation of responsibilities and rights are agreed and documented	Yes	This is a non-commercial study with a single NHS site, where that single NHS organisation is also the study sponsor. Therefore no study agreements are expected.
4.2	Insurance/indemnity arrangements assessed	Yes	No comments
4.3	Financial arrangements assessed	Yes	No application for external funding has been made.
5.1	Compliance with the Data Protection Act and data security issues assessed	Yes	No comments
5.2	CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed	Not Applicable	No comments
5.3	Compliance with any applicable laws or regulations	Yes	No comments
6.1	NHS Research Ethics Committee favourable opinion	Yes	No comments

Section	Assessment Criteria	Compliant with Standards	Comments
	received for applicable studies		
6.2	CTIMPS – Clinical Trials Authorisation (CTA) letter received	Not Applicable	No comments
6.3	Devices – MHRA notice of no objection received	Not Applicable	No comments
6.4	Other regulatory approvals and authorisations received	Not Applicable	No comments

Participating NHS Organisations in England and Wales

This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.
This is a non-commercial study with a single NHS site where that single NHS organisation is also the study sponsor. If this study is subsequently extended to other NHS organisation(s) in England or Wales, an amendment should be submitted, with a Statement of Activities and Schedule of Events for the newly participating NHS organisation(s) in England or Wales. The Chief Investigator or sponsor should share relevant study documents with participating NHS organisations in England and Wales in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office providing the research management function at the participating organisation. Where applicable, the local LCRN contact should also be copied into this correspondence. If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England and Wales which are not provided in IRAS, the HRA or HCRW websites, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net or HCRW at Research-permissions@wales.nhs.uk. We will work with these organisations to achieve a consistent approach to information provision.

Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and Wales, and the minimum expectations for education, training and experience that PIs should meet (where applicable).
A Principal Investigator is expected to be in place at the participating organisation. GCP training is not a generic training expectation, in line with the HRA/HCRW/MHRA statement on training expectations.

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken
No access arrangements are expected as all study activity at the participating NHS organisation will be undertaken by NHS staff who have a contractual relationship with the organisation.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England and Wales to aid study set-up.
The applicant has indicated that they do not intend to apply for inclusion on the NIHR CRN Portfolio.

ii) Confirmation of Research Ethics Committee (REC – Hampshire B) approval

NHS
Health Research Authority
South Central - Hampshire A Research Ethics Committee
Level 3, Block B
Whitefriars
Lewins Mead
Bristol
BS1 2NT

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

22 May 2018

Mr Sunil Ohri
Hamtun House
9a Westrow Road, Banister Park
Southampton
SO15 2NA

Dear Mr Ohri

Study title:	**Predictors of progression of calcific stenosis in patients with Type 2 Diabetes to identify indications and optimal timing to intervention.**
REC reference:	**18/SC/0162**
IRAS project ID:	**240397**

Thank you for your letter of 23 April 2018, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair, along with a Sub-Committee of the REC.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact hra.studyregistration@nhs.net outlining the reasons for your request.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
GP/consultant information sheets or letters [PROCAS - GP Letter v2.0 Highlighted changes]	2.0	24 April 2018
GP/consultant information sheets or letters [PROCAS - GP Letter v2.0]	2.0	24 April 2018
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Summary CV for student [Summary CV - Suresh]	v1.0	15 March 2018
Summary CV for supervisor (student research) [CV - Sunil Ohri]	v1.0	15 March 2018

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

18/SC/0162	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely

pp. Roberts.

Canon Ian Ainsworth-Smith
Chair

Email:nrescommittee.southcentral-hampshirea@nhs.net

Copy to: *Ms Claire Ayling, Research & Development Department (Division D)*

iii) Patient Information Sheet

University Hospital Southampton NHS
NHS Foundation Trust

Patient Information Sheet – Surgical Group
Predictors of Calcific Aortic Stenosis in Diabetes (the PROCAS study)

Study Number: RHM CAR 0541

REC Reference: 18/SC/0162

IRAS Project ID: 240397

Mr Sunil Ohri, Consultant Cardiac Surgeon
D Level North Wing, Mailpoint 46
Southampton General Hospital
Tremona Road
Southampton
SO16 6YD

1. Invitation

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide if you wish to take part. The research team will usually visit you at a later date (prior to your operation) to discuss the study with you again and answer any questions you have before you make a decision on whether or not you wish to participate. If you are having your surgery urgently, we will only invite you to participate if you have had more than six hours to read this information sheet and ask any questions before you undergo surgery.

2. What is the purpose of the study?

The aortic valve is the outlet valve of the heart and is generally made up of three leaflets. Thickening of the leaflets leads to narrowing of the aortic valve and results in failure of the valve to open normally (aortic stenosis). Patients with Type 2 Diabetes are predisposed to a more severe form of this disease. New research shows that several components in human blood may indicate how severe the disease is in humans. To test this, blood tests from participants will be compared with examination of both the aortic valve (once it has been removed) and a small specimen (less than 2 millimetres of heart muscle tissue). In some cases, the heart muscle may perform better following valve replacement. This can be assessed by echocardiography (an ultrasound scan of your heart)

3. Why have I been invited?

You have been invited because you need replacement of your aortic valve. Your surgeon is conducting in the study. The study will aim to enrol 64 patients from

one hospitals; University Hospital Southampton NHS Foundation Trust (Southampton General Hospital) Southampton, UK.

4. Do I have to take part?

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

5. What will happen to me if I take part?

You will undergo a few additional tests. There will be in addition to the blood tests and imaging which you will have as part of your operation, and are detailed below

1. *Several days/weeks before operation* – You will have a **blood test**. An additional 20mililitres (20ml) of blood taken before your operation to test for markers of aortic valve disease.
2. *During your operation* - your surgeon will remove your aortic valve and replace it with a prosthetic valve. Your native, diseased aortic valve is usually discarded. As part of this study, **your native valve will be collected by the research team** to be viewed under a microscope and to perform tests in a laboratory. Your valve will be securely disposed of at the end of the study.
3. *During your operation* - the surgeon will have access to the inner chamber of the heart. As part of the research, we perform a **biopsy of the heart muscle**, where the surgeon will cut a small (2 millimetres) piece of heart muscle from this chamber to be viewed under a microscope. **Potential side-effect of this are explained in Section 9.**
4. *9 months after your operation* - you will be invited for an **echocardiogram scan** (an ultrasound scan of your heart) at University Hospital Southampton to assess both your valve and the function of your heart.
5. *9 months after your operation* - A **blood test** (20 millilitres) of blood will again be taken to test for the same markers of aortic disease as before.

6. What do I have to do?

If you decide to participate there will be no changes to your routine clinical care after your surgery. However, you will be invited to attend the follow-up appointments mentioned above. There will be no particular lifestyle or dietary

restrictions over and above those advised for cardiac surgery patients. Similarly, there are no extra restrictions concerning driving or sport over and above those advised for cardiac surgery patients. You should continue to take all medications prescribed to you as a part of your routine clinical care.

7. **What are the alternatives for diagnosis or treatment?**

If you were not taking part in the study, you would receive the standard treatment for replacement of the aortic valve and the quality of your care would not be affected.

8. **What are the side effects of any treatment received when taking part?**

There are no treatment interventions in this study.

9. **What are the possible disadvantages and risks of taking part?**

During your operation, the only additional procedure that will be performed for this research study is the biopsy of your heart muscle. This procedure is safe and is performed routinely as an outpatient procedure on some patients and is made even more safe as we will be performing it during your heart operation, where any complications can be safely dealt with immediately under controlled conditions. Potential complications are an irregular heart rhythm and bleeding – these are rare. The overall complication rate of myocardial biopsies is **0.0005% (or 1:2000)**.

The additional research-specific blood tests are taken in the same way as a routine blood test and as such, the risks of minor complications such as bruising at the site may occur.

10. **What are the possible benefits of taking part?**

If a condition is discovered during surgery or follow-up of which you were unaware, your surgeon will discuss this with you in exactly the same way as if you were not participating in the study.

11. **What if new information becomes available?**

Sometimes during the course of a research project, new information becomes available. This usually applies to drug treatments rather than surgical operations. If significant new information came to light that has a bearing on the study, the

research team will contact the participants and with a view to providing a consultation and expert clinical recommendation on any necessary action if required.

12. **What happens when the research study stops?**

When the study is completed you will not be required to continue with any study procedures and your care will continue as usual. Your blood samples and all data related to the trial will be securely stored by the research team as per University Hospitals Southampton NHS Trust's Research and Development policy for a period of no more than five (5) years, after which it will be securely destroyed. All human tissue (your native aortic valve and the biopsy specimen of heart muscle) will be stored securely at the laboratory of the Institute of Developmental Sciences at University of Southampton for the duration of the study with only the immediate research team having access to it. Once the trial has been completed, these samples will be securely destroyed in accordance with the Human Tissue Act.

13. **What if something goes wrong?**

The sponsor for this study is University Hospital Southampton, which also provides insurance cover for any harm which may occur as a result of your participation in the trial. The research and surgical team will provide any medical support required as a direct result of any complications that you may experience as a direct result of participating in this study. Independent advice can be obtained the Patient Support Services (PSS) at University Hospital Southampton NHS Foundation Trust (Tel:02381206325, email: patientsupportservices@uhs.nhs.uk).

14. **Will my taking part in this study be kept confidential?**

If you consent to take part in the research, the Clinical Trials Unit of the University Hospital Southampton NHS Foundation Trust for purposes of analysing the results may inspect of your medical records. Your records may also be looked at by people from the regulatory authorities to check that the study is being carried out correctly. Background data relating to you (such as age, gender, height, weight, medical history and clinical blood results) will be stored securely on a password-protected Trust computer in a locked office at University Hospitals Southampton. Research samples (research blood tests and valve and heart tissue) will be transported to the laboratory at the University of Southampton Institute of Developmental Sciences in an anonymised fashion (i.e. with an alphanumeric code which only the research team will have linked to the database at University

Hospitals Southampton. All information that is collected about you during the course of the research will be kept strictly confidential.

15. **What will happen to the results of the research study?**

When the study has been completed and the data analysed, the results will be presented at medical conferences and published in medical journals. Your identity will not be disclosed in any presentation, publication or report. We will notify you of the final results of the study

16. **Who is organising the research?**

This study is being conducted by Dr Suresh Giritharan (MBChB, MRCS) - Research Fellow in Cardiac Surgery - under the Supervision of Mr Sunil Ohri (Consultant Cardiac Surgeon) at the Southampton General Hospital. This study forms part of a higher postgraduate degree in research (DM, Medicine) being undertaken by Dr Suresh Giritharan. The study is being co-ordinated by the Clinical Trials Unit, University Hospitals Southampton (UHS), Southampton. Your doctor is not being paid for you to participate in the study.

17. **Who has reviewed the study?**

This study has been reviewed by the South Central (Hampshire A) Research Ethics Committee and has been given favourable ethical opinion for conduct. The study has also been peer reviewed by experts at Southampton General Hospital.

18. **Contact for Further Information**

If you decide to take part we will ask you to sign a consent form. You will be given a copy of the signed consent form and the patient information sheet. The doctors and nurses involved in this study will be pleased to discuss any questions or concerns you may have. Independent advice can be obtained the Patient Support Services (PSS) at University Hospital Southampton NHS Foundation Trust (Tel:02381 206325, email: patientsupportservices@uhs.nhs.uk). If you do have any questions or concerns about the study please contact Mr. Suresh Giritharan, Principal Investigator (email address: suresh.giritharan@uhs.nhs.uk)

iv) Consent Form

University Hospital Southampton NHS
NHS Foundation Trust

CONSENT FORM – SURGICAL GROUP

Study Title: PRedictors Of Calcific Aortic Stenosis in Diabetes
(The PROCAS Study)

Patient Identification Number :.....
Patient Name:.....
Hospital No: .....
Date of Birth:.....

Insert Addressograph Label Here

	Initials
1. I confirm that I have read and understood the "Patient Information Sheet" version 2.0 dated 24th April 2018 for the above study and have had the opportunity to ask questions.	
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
3. I understand that sections of any of my medical notes and investigations (such as Blood tests, CT scans, echocardiograms and angiograms) may be looked at by responsible individuals from Clinical Trials Unit, Southampton General Hospital or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.	
4. I agree to attend the follow-up clinic appointments	
I consent to undergo blood tests, echocardiography and donation of my aortic valve as part of the research follow-up. I have received written (in the Patient Information Sheet) and verbal information regarding the nature of this procedure.	
5. I consent to allowing the surgeon to perform a biopsy of my heart muscle during my operation. I have been counselled on the nature of this procedure including the associated risks and details of any treatment that may be required should complications occur.	
6. I agree to take part in the above study.	

7. I agree for my GP to be contacted and informed about my participation in the study. ☐

8. I understand that final results of the study will be published in medical journals and notified to participants, containing no personal identifiable information'. ☐

Name of Patient	Date	Signature
Name of Person taking consent	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

v) Publication

Protocol

Aortic Stenosis Prognostication in Patients With Type 2 Diabetes: Protocol for Testing and Validation of a Biomarker-Derived Scoring System

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Abstract

Background: Type 2 diabetes mellitus (T2DM) has been established as an important independent risk factor for aortic stenosis. T2DM patients present with a higher degree of valve calcification and left ventricular dysfunction compared to patients without diabetes. This may be due to an increase in incidence and severity of myocardial fibrosis. Currently, there is no reliable method of determining the optimal timing of intervention for a patient with asymptomatic aortic stenosis or predicting when a patient will become symptomatic. Research into serum biomarkers to predict subclinical onset and track progression of aortic stenosis is hampered by the multimodal nature of the pathological processes ultimately responsible for aortic stenosis.

Objective: The aim of this study is to prove that an approach using a combination of serum biomarkers and the echocardiographic parameter global longitudinal strain (GLS) can be used to establish baseline status of fibrocalcific aortic valve disease, predict rate of progression, and quantitatively assess any regression of these processes following aortic valve replacement in patients with T2DM.

Methods: Validated serum biomarkers for the separate processes of calcification, inflammation, oxidative stress and fibrosis can be used to quantify onset and rate of progression of aortic stenosis. This, in combination with the echocardiographic parameter GLS, can be compared with other objective investigations of calcification and fibrosis with the aim of developing a quick, noninvasive one-stop assessment of aortic stenosis in patients with T2DM. The serum biomarkers BNP (B-type natriuretic peptide), Gal-3 (Galectin-3), GDF-15 (growth differentiation factor-15), sST2 (soluble suppression of tumorigenicity 2), OPG (osteoprotegerin), and microRNA 19b and 21 will be sampled from patients undergoing aortic valve replacement (with and without T2DM), patients with T2DM but without aortic valve disease and healthy volunteers. These patients will also undergo computed tomography (CT) scans for calcium scoring, magnetic resonance imaging (MRI) to quantify myocardial fibrosis, and myocardial strain imaging with speckle-tracking echocardiography. Samples of calcified native aortic valve and a biopsy of ventricular myocardium will be examined histologically to determine the quantity and distribution of calcification and fibrosis, and the secretome of these tissue samples will also be analyzed for levels of the same biomarkers as in the serum samples. All patients will be followed up with in 3 months and 12 months for repeat blood sampling, echocardiography, and CT and MRI imaging to assess disease progression or regression. The results of tissue analysis and CT and MRI scanning will be used to validate the findings of the serum biomarkers and echocardiographic assessment.

Results: Using all of the information gathered throughout the study will yield a ranking scale for use in the clinic, which will provide each patient with a fibrocalcific profile. This can then be used to recommend an optimal time for intervention.

Conclusion: A reliable, validated set of serum biomarkers combined with an inexpensive bedside echocardiographic examination can now form the basis of a one-stop outpatient-based assessment service, which will provide an accurate risk assessment in patients with aortic stenosis at first contact.

International Registered Report Identifier (IRRID): PRR1-10.2196/13186

(JMIR Res Protoc 2019;8(8):e13186) doi: 10.2196/13186

KEYWORDS

aortic stenosis; myocardial fibrosis; type 2 diabetes mellitus; biomarkers; ventricular remodelling; aortic valve replacement

Introduction

Aortic stenosis (AS) remains the most common valvular disease in adults, with a prevalence of 5% in people over the age of 65 years [1]. While type 2 diabetes mellitus (T2DM) has been established as an independent risk factor for this disease, little is understood about the precise nature of this association. Initially, it was attributed to the same inflammatory processes involved in the development of coronary artery disease (CAD) [2]. This theory is now in doubt as modulation of coronary risk factors do not reduce the incidence of aortic stenosis in these patients [3]. Advanced stages of AS warrants surgical aortic valve replacement (AVR) [4]. Despite the aid of echocardiographic parameters allowing for delineation of mild, moderate and severe aortic stenosis, the single prevailing indication for surgical intervention is the severity of symptoms [5].

The physiological narrowing of the valve which limits the outflow of blood is the end result of a complex array of cellular mechanisms that culminate in two distinct but intertwined pathological processes: calcification and fibrosis [6]. These mechanisms are also responsible for the stiffening and loss of elasticity of ventricular muscle due to abnormal deposition of collagen, a process termed myocardial fibrosis [7]. Currently, there is no reliable method of ascertaining if, or how soon, a patient with asymptomatic AS will become symptomatic. Given that these cellular modulations occur insidiously presumably over a duration of time prior to the onset of symptoms it is likely that the ventricular myocardium will have sustained a degree of fibrotic insult prior to identification of the need for aortic valve replacement. This current approach to guiding therapy has the potential pitfall of depriving these patients of the therapeutic effect of early reverse remodeling of the ventricle. This is a process where stiffened, fibrotic myocardium has the potential to regress to its initial elastic, nonhypertrophic state once ventricular afterload (secondary to stiffening by calcification and fibrosis) is reduced by AVR [8].

As the presence of T2DM has been shown to accelerate the progression of aortic stenosis, it is not unreasonable to conclude that patients with T2DM who develop severe aortic stenosis warranting AVR may have more advanced underlying myocardial fibrosis. This may not be amenable to the beneficial phenomenon of reverse-remodeling following replacement of the valve; such T2DM patients may benefit from early AVR. A reliable strategy of tracking the cadence of disease progression and susceptibility to reverse-remodeling is therefore necessary, as this would optimize timing of surgery for high-risk patients. We hypothesize that a method utilizing biomarkers for the tracking of underlying cellular processes culminating in aortic stenosis shows promise in achieving this.

Methods

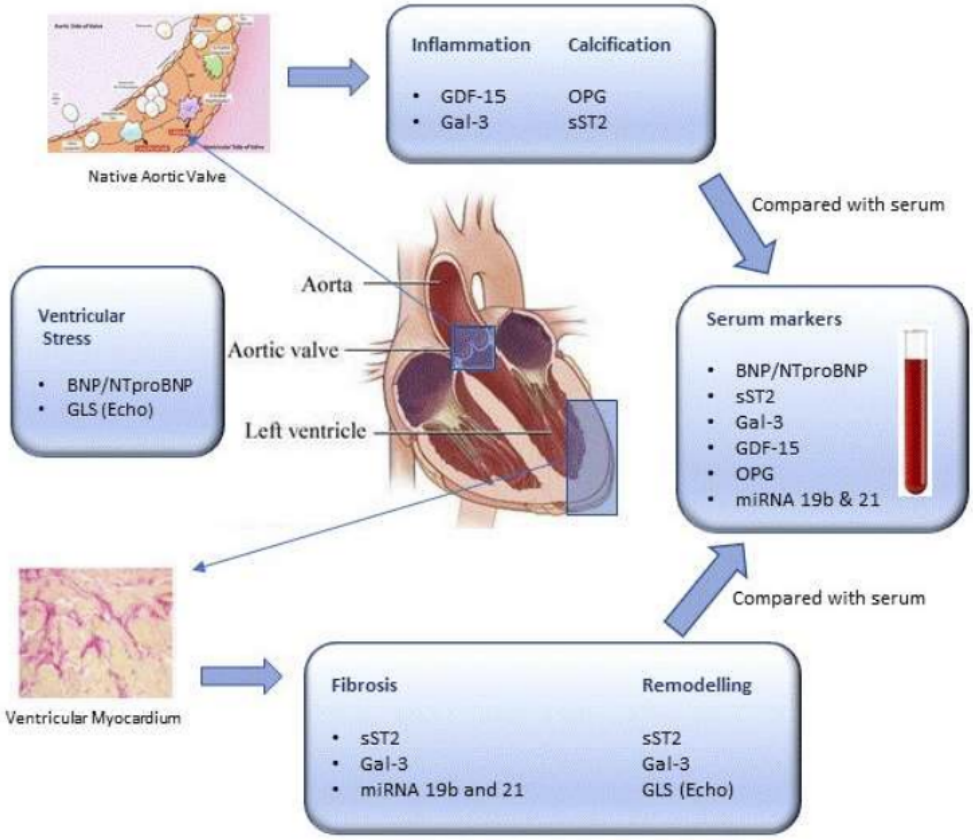
Presentation of the Hypothesis

The identification of a specific serum biomarker which can provide both an insight to the static state of a pathological process and track its progression (and subsequent regression following appropriate intervention) has proved challenging. This is due to the multimodal nature of the mechanisms which ultimately culminate in stiffening of the aortic valve. However, as a result of various studies of calcification and fibrosis in the heart, there is consensus that four core subprocesses are synergistically responsible in achieving the endpoint of aortic stenosis: valve mineralization (calcification), local inflammation, oxidative stress and ventricular fibrosis [9]. Within the domain of each subprocess, several promising biomarkers have shown acceptable specificity [10]. Here, we hypothesize that an approach using a combination of serum biomarkers and the echocardiographic parameter global longitudinal strain (GLS), a functional marker which will be discussed later, can be used to establish baseline status of fibrocalcific aortic valve disease, predict rate of progression and quantitatively assess any regression of these processes following AVR in patients with T2DM.

Serum Biomarkers

Figure 1 provides an overview of the site of expression of relevant biomarkers. BNP (B-type natriuretic peptide), and its prohormone NT-proBNP (n-terminal pro brain natriuretic peptide), are neurohormones and markers of stretching of the heart muscle. BNP is secreted by cardiomyocytes following volume and pressure changes in the left ventricle. NT-proBNP in particular demonstrates an incremental relationship with the severity of aortic stenosis. Although in isolation this biomarker is unable to distinguish between the pathologies of calcification and fibrosis, it is valuable in ascertaining the cumulative consequence of ventricular dysfunction and has predictive value [11].

Figure 1. Location of biomarkers of inflammation, calcification, fibrosis, ventricular stress and remodelling in aortic valve and myocardial tissue which will be compared to levels in serum. BNP, Brain Natriuretic Peptide; Gal-3, Galectin-3; GDF-15, Growth Differentiation Factor 15; GLE, Global Longitudinal Strain; miRNA, micro ribonucleic acid; OPG, Osteoprotegerin; sST2, Soluble ST2.



sST2 (soluble suppression of tumorigenicity 2) is a member of the IL1 (interleukin-1) receptor family and is a marker of ventricular remodeling. sST2 levels increase with myocardial stress and have been demonstrated to correlate quantitatively with myocardial ischemia and heart failure. It is of particular use as it correlates with echocardiographic assessment of diastolic dysfunction and has been demonstrated to correlate with heralding the onset of new symptoms in previously asymptomatic aortic stenosis patients. High levels of sST2 are an independent predictor of mortality following aortic valve replacement surgery [12].

Gal-3 (Galectin-3) is a beta-galactoside-binding lectin and a marker of inflammation and fibrosis of the myocardium, but it is also responsible for other processes such as regulating T-cell function and apoptosis. Gal-3 has a limited role in predicting progression of ventricular stiffening as it is also upregulated in other conditions such as renal dysfunction. However, when considered in tandem with BNP, it has shown to have significant predictive properties in the progression of heart failure [13].

The microRNAs 21 and 19b are specific small RNAs that are 19-25 nucleotides long which are postulated to regulate gene expression related to various cellular mechanisms underpinning cardiovascular function. Although assessment of these micro-RNAs is still in early stages, microRNA-21 has shown good correlation with myocardial fibrosis. MicroRNA-19b has shown promise in determining increased myocardial collagen cross-linking in patients with aortic stenosis [14].

GDF-15 (growth differentiation factor-15) is a stress-responsive cytokine that is sensitive to inflammatory changes and apoptosis. It is abundant in cardiomyocytes, adipocytes and macrophages and has demonstrated good predictive value in heart failure, vascular calcification and endothelial dysfunction. Like Gal-3, it is upregulated in renal dysfunction and additionally in obesity and insulin-resistant states such as diabetes. Although recent work has established GDF-15 as a useful prognostic and diagnostic marker of cardiometabolic disease in patients with diabetes, reference ranges correlating to severity have not yet been established [15].

OPG (osteoprotegerin) is a member of the tumor necrosis factor receptor family and is expressed in early arteriosclerotic lesions and vascular smooth muscle cells. It has an important role in neocalcification, acting as a decoy receptor by binding to the RANKL (receptor activator kappa-B ligand) nuclear factor and inhibiting the receptor activity of RANK (receptor-activated kappa-B). This thus inhibits osteoclastic activity, which ultimately leads to calcification. Modulation of this pathway

by the action of anti-Ghrelin antibodies suppressing OPG expression is of particular interest in seceding calcification [16].

Global Longitudinal Strain as a Complimentary Functional Marker

Speckle-tracing echocardiography is a relatively novel echocardiographic technique which has been shown to be more sensitive than conventional assessments of ejection fraction (EF) in the assessment of minute, regional wall motion abnormalities (RWMA) and left ventricular function. It correlates well with the presence and magnitude of myocardial fibrosis when compared alongside cardiac magnetic resonance, as assessed through T1-mapping and late gadolinium enhancement as quantification techniques. This noninvasive imaging modality can serve as an additional functional marker alongside the serum biomarkers mentioned above in the assessment of severity of aortic calcification and in the evaluation of reverse-remodeling postoperatively [17].

Testing the Hypothesis

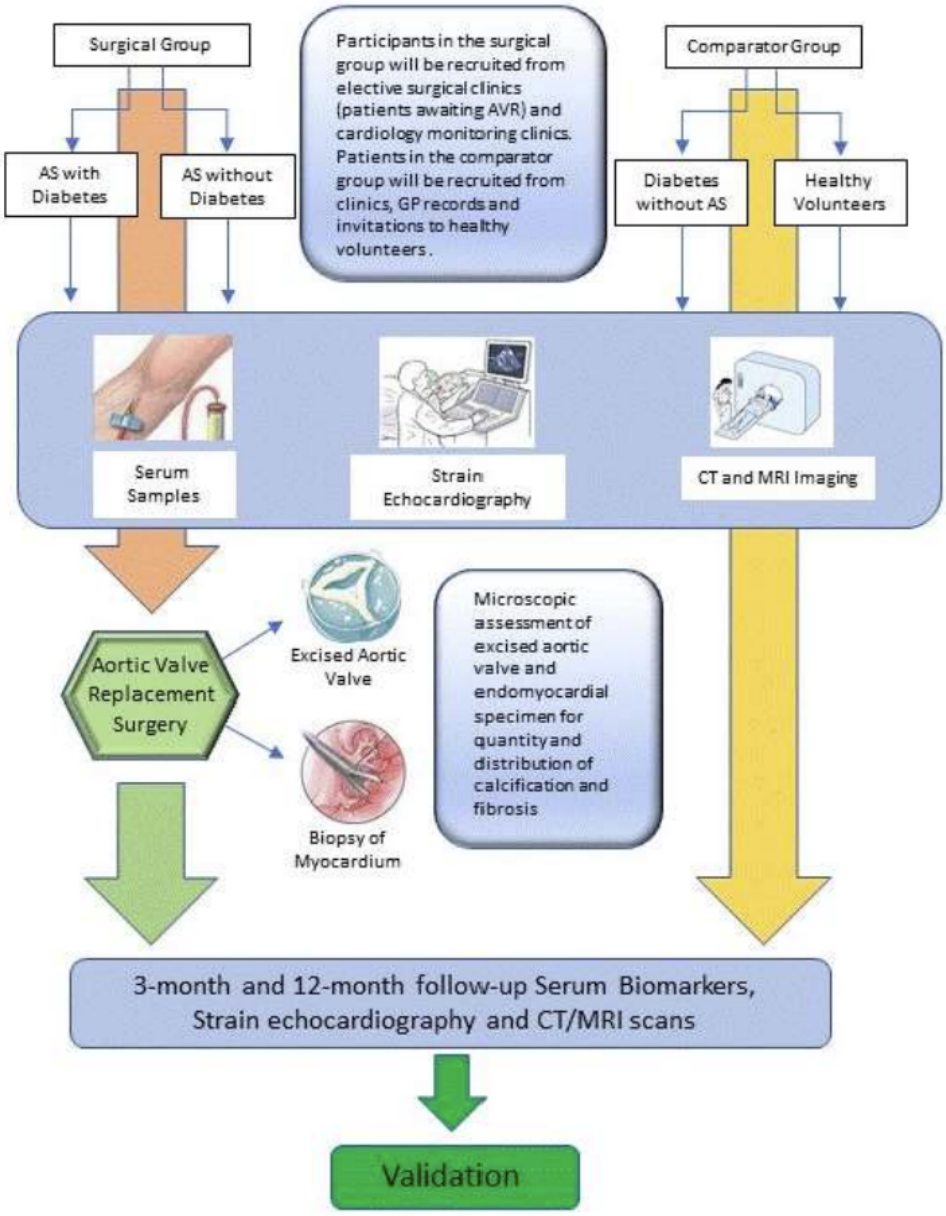
Figure 2 outlines the process of biomarker testing in the relevant patient cohorts. Gold standard assessments of aortic valve calcification and myocardial fibrosis are direct microscopic inspection of the excised aortic valve, following AVR, and a biopsy specimen of the ventricular myocardium, respectively [18]. Noninvasive imaging techniques done preoperatively, Computed Tomographic (CT) Calcium Scoring for aortic valve calcification and Late Gadolinium Enhancement magnetic resonance imaging (MRI) for myocardial fibrosis, will corroborate the findings of direct inspection. These results will be used to calibrate serum biomarkers and echocardiographic findings for the subset of patients with severe AS undergoing surgery.

Healthy volunteers and patients with milder, asymptomatic forms of the disease will be recruited from outpatient cardiology surveillance clinics to undergo serum biomarker sampling and strain echocardiograms. They will then undergo CT and MRI scans to investigate the relevance of these markers against objective imaging findings. This will enable the establishment of baseline levels in healthy volunteers and grading of the markers in mild, moderate and severe forms of disease.

Finally, postoperative patients will be followed up with in 3 months and 12 months following surgery where they will once again undergo serum biomarker sampling and strain echocardiography to assess for evidence of reverse-remodeling. Again, CT scans for calcium scoring and MRI scans will be repeated to validate the significance of any downregulation of these biomarkers.

The combination of biomarker and echocardiographic data encompassing the entire spectrum of the disease from healthy volunteers to patients with severe disease as well as disease monitoring in postoperative patients will yield a ranking scale for use in the clinic. This scale, once validated for reproducibility in an appropriately powered study, will provide each patient with a fibrocalcific profile which can then be used to recommend an optimal time for intervention.

Figure 2. Testing the hypothesis. Preoperative serum biomarkers and echocardiography will be compared to CT and MRI imaging to quantify calcification and fibrosis. Samples of valve and myocardial tissue will be examined for calcification and fibrosis and compared to serum biomarkers. Follow-up investigations will assess if upregulation or downregulation of biomarkers show reliable clinical correlation with disease progression or regression. AS: aortic stenosis; AVR: aortic valve replacement; CT: computed tomography; MRI: magnetic resonance imaging.



Results

Enrollment has not commenced for this study. Ethical approval was obtained from South Central - Hampshire A Research Ethics Committee (United Kingdom – REC: 18/SC/1062, IRAS: 024397). Funding was obtained from the Wessex Heart Surgery Charitable Fund (registered charity #1051543).

Discussion

A reliable, validated set of serum biomarkers combined with an inexpensive bedside echocardiographic examination can now form the basis of a one-stop outpatient-based assessment service in providing an accurate risk assessment in patients with AS at first contact. In patients with diabetes, the role of such a service can be expanded to an essential screening service given the less favorable prognosis of aortic stenosis in this demographic.

Acknowledgments

This manuscript received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Authors' Contributions

SG is responsible for the conceptualization of the hypothesis. SO provided guidance on the choice of biomarkers. FC and CT recommended appropriate laboratory techniques for analysis of the biomarkers. KS and SD designed the experimental model for testing the hypothesis. All authors provided final critical review of the manuscript.

Conflicts of Interest

None declared.

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Abbreviations

AS: aortic stenosis
AVR: aortic valve replacement
BNP: brain natriuretic peptide
CAD: coronary artery disease
CT: computed tomography
EF: ejection fraction
Gal-3: Galectin 3
GDF-15: growth differentiation factor 15
GLS: global longitudinal strain
IL1: Interleukin-1
MRI: magnetic resonance imaging
NT-proBNP: n-terminal pro brain natriuretic peptide
OPG: osteoprotegerin
RANKL: receptor-activated kappa B ligand
RNA: ribonucleic acid
RWMA: regional wall motion abnormality
sST2: soluble suppression of tumorigenicity 2
T2DM: type 2 diabetes mellitus

Edited by G Eysenbach; submitted 18.12.18; peer-reviewed by M Banovic, A Korchi; comments to author 14.04.19; revised version received 27.04.19; accepted 27.04.19; published 12.08.19

Please cite as:
Giritharan S, Cagampang F, Torrens C, Salhiyyah K, Duggan S, Ohri S
Aortic Stenosis Prognostication in Patients With Type 2 Diabetes: Protocol for Testing and Validation of a Biomarker-Derived Scoring System
JMIR Res Protoc 2019;8(8):e13186
URL: https://www.researchprotocols.org/2019/8/e13186/
doi: 10.2196/13186
PMID: 31407670