A Journey Through The Thoracic Aorta:

From Root To Arch

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ABSTRACT

This thesis investigated the clinical and pathological outcomes of disease of the aortic valve and the ascending aorta, including the aortic root.

Purpose:

The aortic valve and proximal aorta are the anatomical origin of the systemic circulation and pathology in this area can be catastrophic. Despite this, there is limited knowledge of the pathophysiology of proximal aortic aneurysms and patient outcomes for treatment strategies of aortic valve disease. The purpose of this thesis is to strengthen the knowledge of pathology affecting the thoracic aorta so that it can help guide clinical management in the future.

Aims:

This thesis aims to investigate the effects of pathology on the aortic valve and thoracic ascending aorta through determination of:

1. The pathophysiology of proximal aortic aneurysms, specifically focusing on evaluating aortic root and ascending aortic aneurysm in relation to (a) histology (Chapter 2) and (b) propensity to aortic rupture in pigs in-vitro (Chapter 3) and in-vivo (Chapter 4) models; and

2. Comparing SAVR & TAVR in relation to (a) clinical outcomes (Chapter 5) and (b) patient-related outcomes (Chapter 6).

Methods:

Chapter 2, focused on histological analysis of aneurysmal aortas, and involved laboratory preparation of human aneurysmal and non-aneurysmal tissue and analysis utilizing histological and immunohistochemistry techniques.

Chapter 3, which focused on a laboratory pig model in ascending aorta and aortic root rupture, involved laboratory preparation of pig non-aneurysmal samples, and utilised a unique pressure testing apparatus, to determine the maximal stress the root and ascending aorta can withstand prior to rupture. This was a pilot study for Chapter 4.

Chapter 4, which focused on a live pig model in ascending aorta and aortic root rupture, involved placement of live pigs on cardiopulmonary bypass, and determination of maximal aortic pressures prior to rupture or failure of the aorta clinically and radiologically using 4D flow MRI. This ruptured tissue was then analysed utilising histological and immunohistochemistry techniques.

Chapter 5 which focused on clinical outcomes of aortic valve surgery, utilised the ANZSCTS national database from Monash Health, incorporating data collection and analysis from 2001 to 2019.

Chapter 6 which focused on the patient-related outcomes following aortic valve surgery, involved the use of validated questionnaires of patients over a 12-month period following their surgery. Specific outcomes measured included frailty, depression, angina, and quality of life.

Results and Discussion:

Chapter 2, 3, and 4 focus on the comparisons in structure between two anatomical regions of the aorta, while Chapter 5 and 6 focus on comparisons in approach between two methods of aortic valve replacement. All Chapters give us valuable knowledge as to how we can manage aortic pathology not only during surgery, but also during the patient's perioperative journey.

The aortic root is the most susceptible region of the thoracic aorta and is predisposed to progression of pathology and rupture in clinical testing. The aortic root is more vulnerable to high pressures, further exacerbated by aneurysmal changes, supported by both microscopic and macroscopic characteristics, while the ascending aorta retains its resilience in comparison. This identified a difference between the aortic root and ascending aorta not only in known anatomical and physiological form, but in each areas ability to maintain its integrity in severe stress and aneurysmal pathological change.

With respect to outcomes post aortic valve replacement, the ANZSCTS database showed no difference in composite endpoints of mortality and stroke between Surgical aortic valve replacement (SAVR) and Transcatheter aortic valve replacement (TAVR), while the degree of morbidity (complete heart block requiring pacemaker and vascular complications) was more prevalent in TAVR groups. In contrast, quality of life, depression, angina, and frailty consecutively measured over 12 months, showed significant improvement in both SAVR and TAVR groups, and an obvious benefit to these measures in all patients requiring intervention for aortic stenosis. When these two groups (SAVR and TAVR) were matched, clinically relevant preoperative variables were identified as being predictive of early mortality.

Conclusions:

The aortic root differs to the ascending aorta under maximal stress and in response to pathological change. Following further clinical testing and human trials, consideration should be for surgical management of these structures as separate entities.

Transcatheter approaches are evolving with improved outcomes in large scale randomised trials supported by our findings of composite primary end points, as well as comparable improvement in quality of life, angina, depression, and frailty with surgical groups. Clinically significant morbidity in the form of vascular and electrophysiological complications remain high, and this should be a focus of ongoing long term clinical trials before an absolute incorporation of this technique for all patients with aortic stenosis.

Recommendations:

This unique analysis offers a new perspective of root and ascending aorta dilatation with strong clinical implications. These two structures deserve new and different management.

National databases reporting on outcomes in aortic valve surgery should consider combining databases regardless of transcatheter or open surgical approach, to allow for a comprehensive and accurate representation of morbidity and mortality outcomes.

Longer term analysis of these morbidity results should guide clinical guidelines as to the appropriate use of these techniques in aortic valve disease, and the utilisation of combined surgical and physician teams in performing these procedures.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree. I acknowledge that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

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Timothy L. Surman
May 2022

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I would like to acknowledge and thank Mr Michael Worthington for his mentorship, support, and guidance in the development and shaping of many of the research chapters in this thesis. As Director of the D'Arcy Sutherland Cardiothoracic Surgical Unit at the Royal Adelaide Hospital, he has incorporated much of the available funding to the fulfillment of many of these research chapters presented here.

I would like to acknowledge and thank Dr John Matthew Abrahams for his friendship, support, guidance, and patience throughout this process. John has helped shape and develop many of the ideas in the early stages of chapter development. He has most importantly been an ongoing support in the statistical analysis, publication editing, and thesis structure development over the last two years. John has been the first point of contact in many of the above publications, and is second author in multiple publications for a clear and absolute reason. I thank John for his ongoing friendship and support.

DEDICATION

I would like to dedicate this thesis to my family. To my parents, Ashley and Mary Surman, and my sister Hayley Surman. Their support throughout this process was ongoing and invaluable.

"Wherever the art of medicine is loved, there is also a love of humanity" - Hippocrates

PUBLICATIONS ARISING FROM THIS THESIS

1. **Surman TL**, Abrahams JM, O'Rourke D, Reynolds KJ, Edwards J, Worthington MG, Beltrame JB (2020). The functional limits of the aneurysmal aortic root. A unique pressure testing apparatus. The Journal of Cardiothoracic Surgery; 15: 259.

2. **Surman TL**, Abrahams JM, Manavis J, Finnie J, O'Rourke D, Reynolds KJ, Edwards J, Worthington MG, Beltrame JB (2021). Histological regional analysis of the aortic root and thoracic ascending aorta: A complete analysis of aneurysms from root to arch. The Journal of Cardiothoracic Surgery; 16: 255.

3. **Surman TL,** Abrahams JM, Manavis J, Finnie J, Christou C, Williams GK, Walls A, Frantzis P, Adams M, Edwards JE, Worthington MG, Beltrame JB (2021). The susceptibility of the aortic root: porcine aortic rupture testing under cardiopulmonary bypass. The Journal of Cardiothoracic Surgery; 16: 283.

4. **Surman TL,** Abrahams JM, Williams-Spence J, Edwards JE, Worthington MG, Beltrame JB, Smith JA (2022). Clinical outcomes in surgical and transcatheter aortic valve replacement. An ANZSCTS database review 2001-2019. Heart, Lung and Circulation, May 2022 https://doi.org/10.1016/j.hlc.2022.04.047.

5. **Surman TL**, Abrahams JM, Kim J, Surman HE, Roberts-Thomson R, Edwards JE, Worthington MG, Beltrame JB (2022). Quality of life and frailty outcomes following surgical and transcatheter aortic valve replacement. The Journal of Cardiothoracic Surgery; 17: 113.

ABBREVIATIONS

ECG – Electrocardiogram

CT – Computed Tomography

MRI – Magnetic resonance imaging

SOV – Sinuses of Valsalva

BAV – Bicuspid Aortic Valve

LVOT – Left ventricular outflow tract

TAVR – Transcatheter aortic valve replacement

SAVR – Surgical aortic valve replacement

AV – Aortic valve

STJ – Sino tubular Junction

PAU – Penetrating aortic ulcer

TAA – Thoracic aortic aneurysm

LDS – Loeys Dietz Syndrome

EDS – Ehlers Danlos Syndrome

TA- Truncus Arteriosus

TAAD – Type A Aortic Dissection

ARR – Aortic root replacement

VSRR – Valve sparing root reimplantation/repair

NC – Non-coronary

LC – Left coronary

RC – Right coronary

H&E – Hematoxylin and Eosin

EVG – Elastic Van-Gieson

CD – Colour deconvolution

MT – Masson's Trichrome

RGB – Red, Green, Blue

CMD – Cystic Medionecrosis

μm - Micro-metres

VK - Von Kossa

H2O2 - Hydrogen peroxide

PBS - Phosphate buffered saline

DAB - Diaminobenzidinetetrahydrochloride

PAS - Periodic acid Schiff stain

ROI - Region of interest

IHC - Immunohistochemistry

CVA - Cerebrovascular accident

SAHMRI - South Australian Health and Medical Research Institute

PIRL - Preclinical, Imaging, and Research Laboratories

IRAD - International Registry of Aortic Dissections

Dp/dT – Derivative of pressure over time as a measure of cardiac contraction

3D – 3-dimensional

4D – 4-dimensional

WSS – Wall sheer stress

CPB – Cardiopulmonary bypass

ROSC – Return of spontaneous circulation

MMP – Matrix metallopeptidases

TIMP – Tissue inhibitor of metalloproteinases

PGD – Primary graft dysfunction

FiO2 – Fraction of inspired oxygen

SvO2 – Mixed venous oxygen saturation

PaO2 – Partial pressure of oxygen

SVC – Superior Vena Cava

WIP – Work in progress

ANZSCTS – Australian and New Zealand Society of Cardiothoracic Surgeons

HREC – Human research ethics committee

CALHN – Central Adelaide Local Healthcare Network

DSWI – Deep sternal wound infection

V/Q – Ventilation and perfusion

CRP – C-reactive protein

ESR – Erythrocyte sedimentation rate

GIT – Gastrointestinal tract

GI – Gastrointestinal

ATSI – Aboriginal or Torres Strait Islander

BMI – Body mass index

BSA – Body surface area

EoA –Effective orifice area

PPM – Permanent pacemaker

NYHA – New York Heat Association

LVEF – Left ventricular ejection fraction

MI – Myocardial infarction

CCF – Congestive cardiac failure

IE – Infective endocarditis

CAD – Coronary artery disease

RCT – Randomised Control Trial

AR – Aortic regurgitation

AF – Atrial fibrillation

KPI – Key performance indicator

PROM – Patient related outcome measure

CABG – Coronary artery bypass grafting

PCI – Percutaneous coronary intervention

eGFR – Estimated glomerular filtration rate

TIA – Transient ischemic attack

PVD – Peripheral vascular disease

COPD – Chronic obstructive pulmonary disease

HTN – Hypertension

AVA – Aortic valve area

STS – Society of Thoracic Surgery

QOL – Quality of life

EFT – Essential Frailty Toolset

SAQ – Seattle angina questionnaire

VAS – Visual analogue scale

PTCA – Percutaneous transluminal coronary angioplasty

KCCQ – Kansa City Cardiomyopathy Questionnaire

Pa – Pascal

IU – International units

ACEI – Angiotensin converting enzyme inhibitor

ARB – Angiotensin receptor blocker

ATA – Ascending thoracic aorta

AHF – Anterior heart field

MEF - Myocyte-specific enhancer factor

BMF - Bone morphogenetic protein

OFT – Outflow tract

NOS - Nitric oxide synthase

AP – Aortopulmonary

PV – Pulmonary valve

cTnnt - Cardiac muscle troponin T

SHF – Second heart field

NOTCH - Notch homolog 1, translocation-associated

SMAD - Mothers against decapentaplegic homolog/SMAD family member

GATA – GATA-binding factor

ROBO - Roundabout homolog

MAT - Methionine Adenosyltransferase

ADAMTS - A disintegrin and metalloproteinase with thrombospondin motifs

BRG - ATP-dependent chromatin remodeler SMARCA4

ROCK - Rho Associated Coiled-Coil Containing Protein Kinase

ALK - Activin receptor-like kinase

JAG - Jagged canonical notch ligand

KROX20 - Early growth response 2 (EGR)

ACTA2 - Actin alpha 2

TGFBR1 - Transforming growth factor beta receptor 1

FBN1 - Fibrillin 1

CHAPTER ONE

Introduction

1.1 Development

1.1.1 Embryological development of the aortic root apparatus and thoracic aorta

The ascending aorta and aortic root apparatus have unique embryological development. The most proximal regions of the thoracic aorta (root) have the most complex embryological development and therefore exhibit significant anomalies. Henle was the first to introduce and define the term 'arterial root' to replace the term 'arterial ring' [1, 2]. The aortic root segment begins at the aortic valve (AV) annulus and extends to the sinotubular junction (STJ). It includes the AV, coronary origins, and the sinuses of Valsalva (SOV) [3]. The aortic root complex has been described as composed of three-elliptical rings and one crown-like ring: the virtual basal annulus, the anatomic annulus, the STJ, and a crown-like ring demarcated by the hinges of the leaflets [4] (Figure 1.1). Therefore, the aortic root was very early distinguished as a unique anatomical entity.

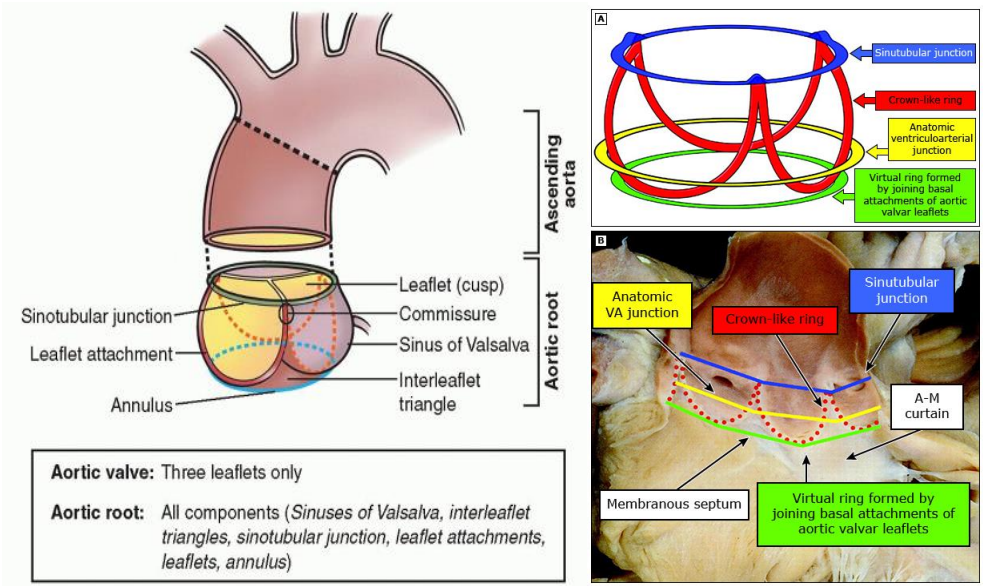

Figure 1.1: The illustration on the left shows proposed nomenclature for the aortic root elements [3]. The image on the right shows the anatomical components from a tissue specimen of the aortic valvular complex [4].

Several events sculpt the components of the aortic root into a precise geometrical orientation that ensures optimal structure and function. The order and sequence of these precise events is described below (Figure 1.2).

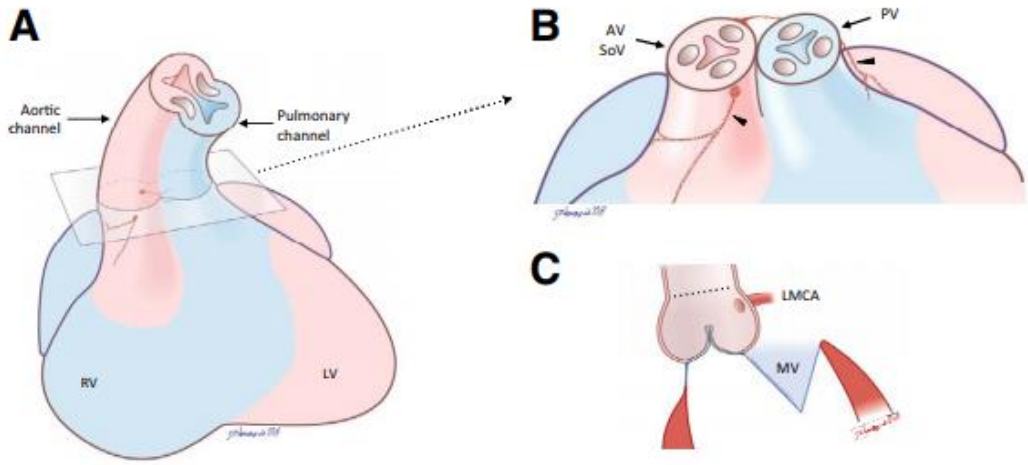

Figure 1.2: Diagrammatic simplification of the embryologic development of the AV and pulmonary valve, and truncus arteriosus (TA) division leading to formation of an aortic and a pulmonary artery channel [5].

The heart begins as a single tube that separates into two tubes and twists onto itself. Cells from the primary cardiac crescent form the primary heart tube. Cells from a second cardiogenic area populate the outflow tract and aortic arches [6].

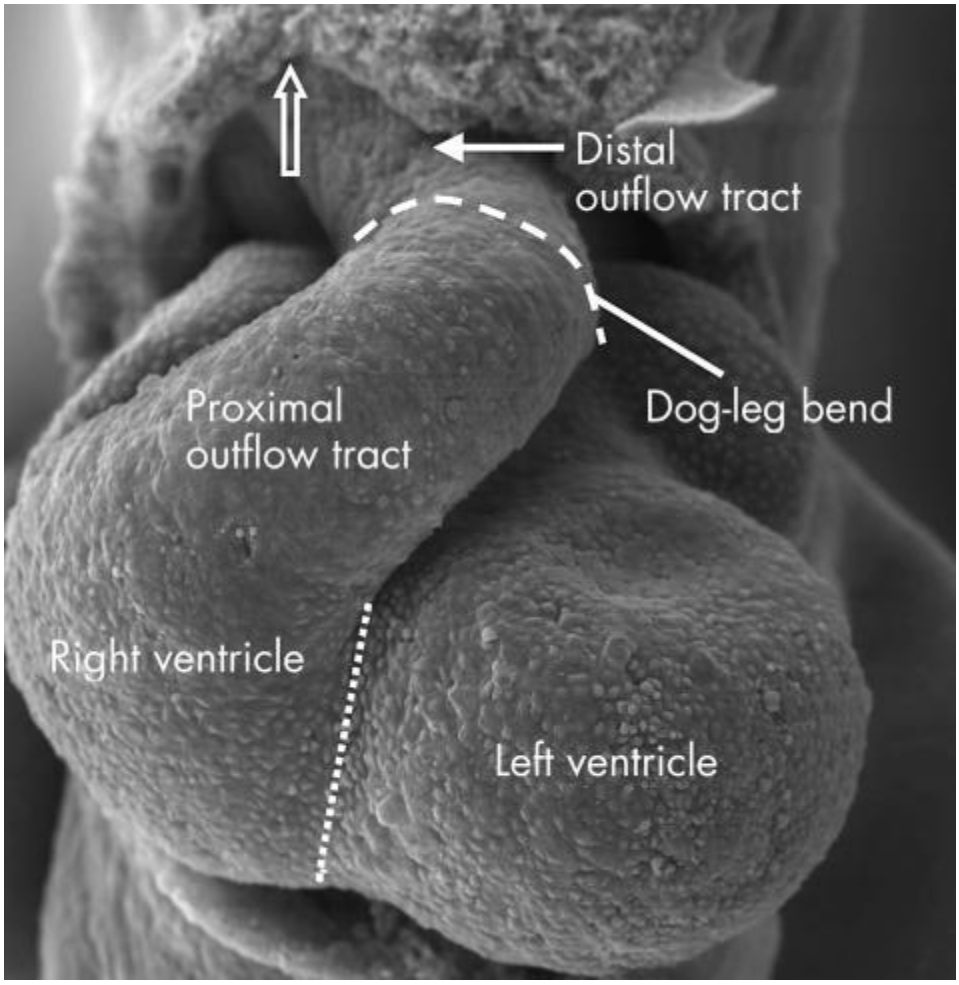

Figure 1.3: Scanning electron micrograph showing the heart from a mouse with 42 somites. The outflow tract is supported by the right ventricle, with the interventricular groove delineated by the dotted line. The outflow tract is divided into proximal and distal ends by the characteristic bend (dashed line) [6]**.**

After rightward looping of the heart, the extracellular matrix (cardiac jelly) overlying the future AV canal and outflow tract expands into swellings known as the cardiac cushions [7]. The outflow tract then divides into proximal and distal portions. Two further intercalated cushions grow in the opposite quadrants of the common outflow tract. The formation of cavities in the distal parts of the proximal cushions as well as the intercalated cushions produce the early cells of the arterial valvular leaflets and sinuses [7]. These structures lie immediately upstream to the developing STJ. Each of the two fused cushions form one sinus and leaflet of the aortic valve, together with the adjacent sinus and leaflet of the pulmonary valve [7] (Figure 1.3 and 1.4).

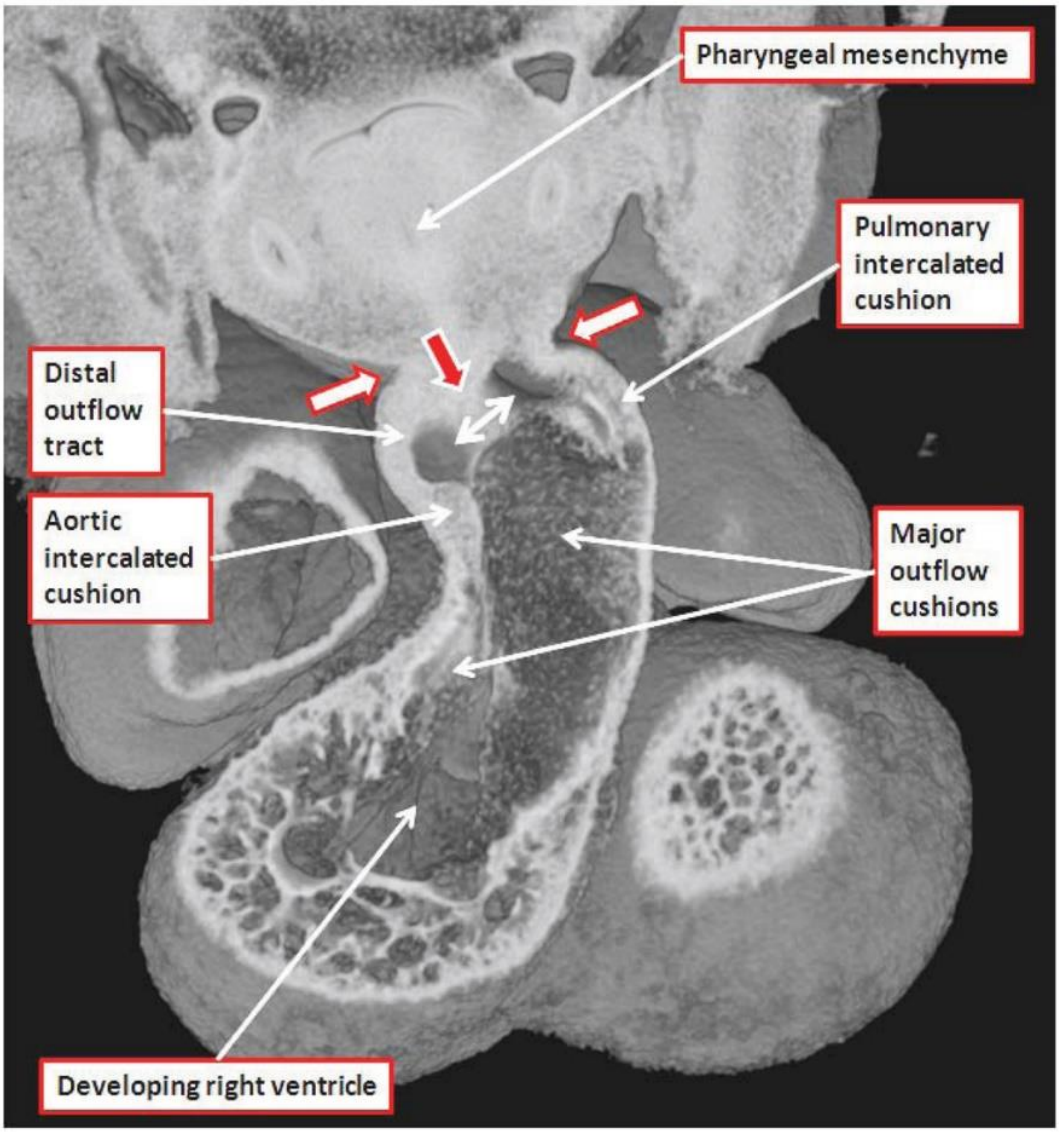

Figure 1.4: Developing mouse through the long axis showing the dogleg bend within the outflow tract, and how the intercalated cushions are appearing at the inner and outer angles of the dogleg [7].

1.1.2 Neural crest cell role in development of the aortic valve

Semilunar valve development is distinguished from atrioventricular valve development by the infiltration of migrating neural crest, which orchestrates important aspects of outflow tract septation and aortic arch artery remodelling [8].

Cardiac neural crest cells delaminate from the dorsal neural tube at approximately embryonic day 8.5 (E8.5) in the mouse and migrate through the pharyngeal arches on their way to the forming heart. Before entering the cardiac outflow tract at approximately E10, neural crest is in close

apposition to second heart field mesoderm. Second heart precursors are characterised by expression of Islet1 and are labelled by transgenic mice that utilise a specific "anterior heart field" (AHF) enhancer of the Mef2c locus. Second heart precursors contribute primarily to myocardium in the right ventricle and outflow tract and to some smooth muscle and endothelial derivatives [8].

The primitive myocardium secretes factors, such as Bmp2, in the cardiac jelly that induce the transition of endothelial cells into mesenchymal cells (EMT). This process results in an invasion of endothelial-derived mesenchymal cells into the cardiac jelly. In the cardiac outflow tract (OFT), EMT results in the formation of a septal and a parietal cushion, the primordia of the myocardial OFT septum and the semilunar valves. Defects in cardiac jelly synthesis result in severely hypoplastic cushions due to failed EMT. Failure of EMT has been shown to result in BAVs. BAV formation in Nos3−/− has also been suggested to be caused by early defects in EMT resulting in reduced mesenchyme populations in the OFT cushions [9].

Migration of cardiac neural crest cells from the neuroectoderm into the OFT cushions induces the formation of the aortopulmonary (AP) septum, through division of the OFT into the aortic and pulmonary orifice. Proximally, the right and left OFT subsequently forms. The parietal cushion gives rise to the right-facing leaflets of the aortic and the pulmonary valve, while the septal cushion will develop into the left-facing leaflets of both valves. Finally, the non-facing aortic leaflet and pulmonary leaflet are considered to be derived from separately developing intercalated cushions on the posterior and anterior sides of the OFT, respectively [9, 10]. In coordination with the fusion of the major OFT cushions, the intercalated cushions develop at right angles to the midline fusion, and give rise to the non-coronary cusp of the AV and anterior cusp of the PV. In the developing AV, formation of the intercalated cushion involves invagination of the endocardial lining into the right coronary cusp prevalvular cushion; however, the majority of the cells that contribute to the intercalated cushions are derived from the cTnnt2-Cre myocardial lineage [11].

Neural crest, endothelial, epicardial cell lineages and second heart field (SHF) – derived cells contribute to both the ascending aorta, aortic valve, and the various components of the aortic root (valvular leaflets, annulus, SOV) [9, 12].

Although many events have been defined in AV formation, the stage, cell type, lineage, and molecular signalling events that generate BAVs largely are unknown. Moreover, the combination of BAV-linked human mutations that have been discovered to date, including NOTCH1, SMAD6, GATA4, GATA5, GATA6, ROBO4, MAT2A, and ADAMTS19, represent a relatively small number of BAV patients, leaving the genetic origins for the majority of individuals with BAV, unknown [11].

1.2 ` Anatomy

1.2.1 Anatomy of the thoracic aorta, aortic root, and aortic valve

1.2.1.1 Anatomy of the ascending aorta

The direct continuation of the left ventricle towards the thoracic aorta is the left ventricular outflow tract (LVOT). The LVOT terminates at the AV annulus and start of the aortic root, and is replaced as the ascending thoracic aorta. This pathway allows for the passage of blood into the systemic circulation. The AV annulus is the most distal limit of the LVOT. Just above the valve leaflets, the aorta gives off the left and right main coronary arteries that run along coronary grooves of the heart. The ascending aorta is the part of the aorta between the STJ (upper limit of the aortic root) and the origin of the first arch vessel (brachiocephalic trunk or artery), and extends approximately to the level of the 4th thoracic (T4) vertebral body where it then becomes the aortic arch [13] (Figure 1.5).

Normally, the proximal aorta lies posterior and to the right of the pulmonary artery and is typically 2.5–3.5 cm in diameter. The transverse and descending thoracic aorta are frequently slightly narrower than the ascending aorta, with diameters rarely greater than 2.5 cm in normal individuals [14].

The normal diameter of the aortic root and ascending aorta is influenced by patient age, gender, and body surface area. In 3431 Framingham Heart Study participants, ECG-gated CT showed mean diameter of 34.1 ± 3.9 mm for the proximal thoracic aorta for men and 31.9 ± 3.5 mm for women. Similarly, a study on SOV diameter in adults demonstrated that mean diameter in end-diastole is 3.2 ± 0.6 cm for men and 2.9 ± 0.5 cm for women. Due to variations in size with patient age, gender, and body surface area, having a single diameter cut off for abnormal diameter would be inaccurate. The traditionally accepted values for the upper limits of normal diameter for SOV and the STJ are 4 cm and 3.6 cm for males and 3.6 cm and 3.2 cm for females respectively [15].

Variations in aortic measurements are noted in Marfan syndrome (MFS), which is one of the most common inherited disorders contributing to thoracic aortic aneurysms, where aortic enlargement is generally maximal at the SOV. This pattern is however also seen in patients without Marfan phenotype. In patients with Bicuspid Aortic Valve (BAV), three enlargement patterns are described, according to whether the maximal aortic diameter is at the level of the SOV, the supracoronary ascending aorta, or the STJ level (cylindrical shape). It is described that there is a relationship between the morphology of the ascending aorta and the valve fusion pattern [16].

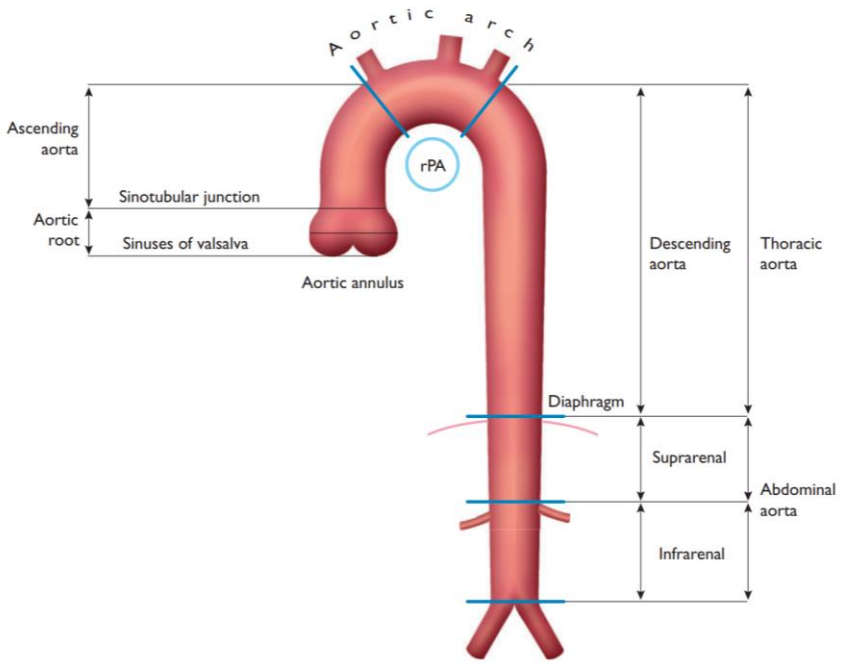

Figure 1.5: Anatomical segments of the aortic root, ascending aorta and descending aorta derived from the 2014 ESC Guidelines on the diagnosis and treatment of aortic diseases [17].

1.1.2.2 Anatomy of the aortic valve and root

The aortic root may be defined as the portion of the LVOT which supports the leaflets of the AV, delineated by the sinotubular ridge superiorly and the bases of the valve leaflets inferiorly. It comprises the sinuses, the AV leaflets, the commissures, and the interleaflet triangles, all of which will be described in detail (Figure 1.1 and 1.6).

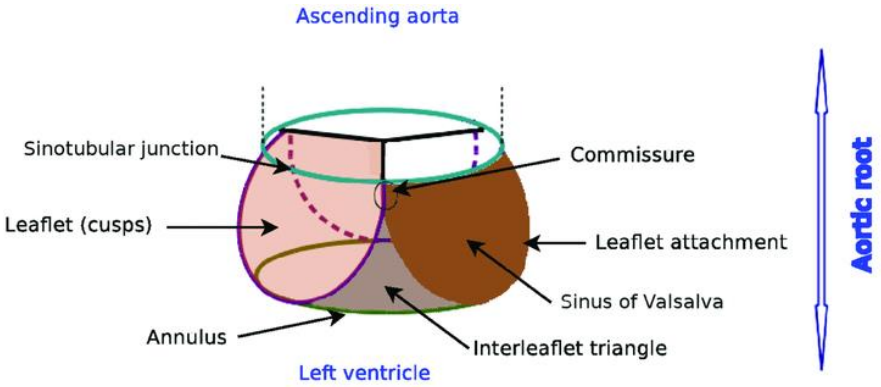

Figure 1.6: The aortic root structure in detail. Annulus, leaflets, leaflet attachment, STJ, interleaflet triangle and SOV are the different components of the aortic root. The AV consists of the three leaflets only [18].

Leaflets

The three leaflets form the AV and provide its sealing mechanism. Leaflets are composed of three main components:

1. The free margin, with a thickened circular node (nodule of Arantius), which provides the coaptation area to the corresponding leaflets.
2. The "belly" of the leaflet.
3. The basal parts of the leaflet or "leaflet attachments".

The AV leaflets form the haemodynamic junction and physical boundary between the left ventricle and the aorta [3] (Figure 1.7).

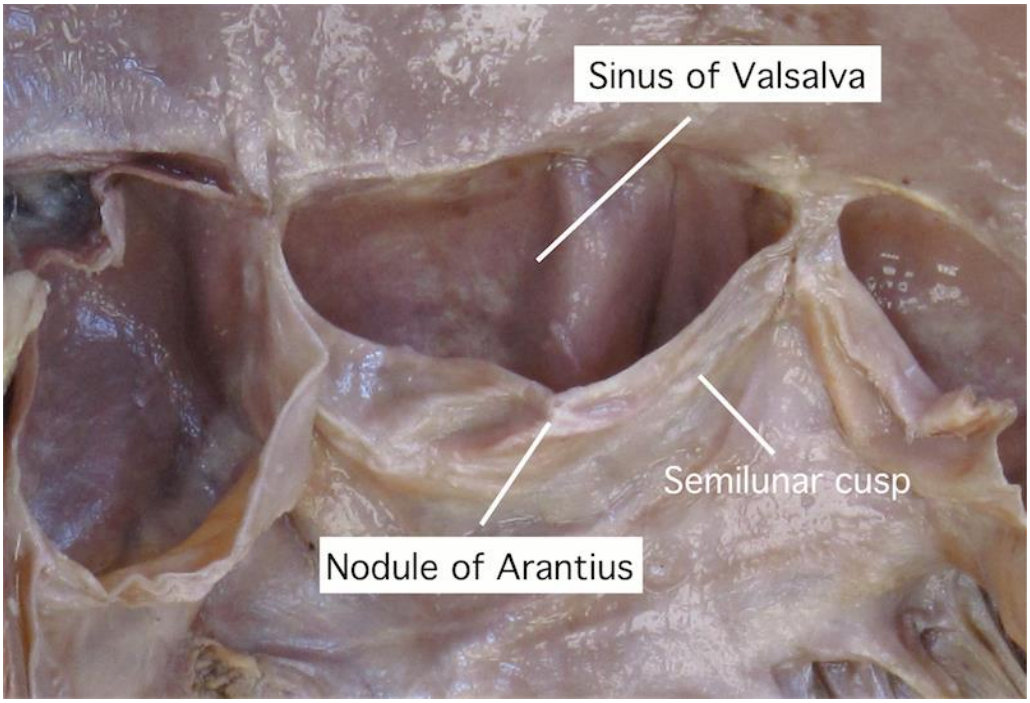

Figure 1.7: Free margin of the non-coronary cusp leaflet with nodule of Arantius as labelled in a pathological human specimen [19].

Leaflet attachments

As the leaflet attachments insert in the wall of the aortic root, they form a crown shaped, thick fibrous structure, often termed the "annulus". The points where the leaflet attachments run parallel - distally towards the ascending aorta - are called the commissures [3] (Figure 1.8).

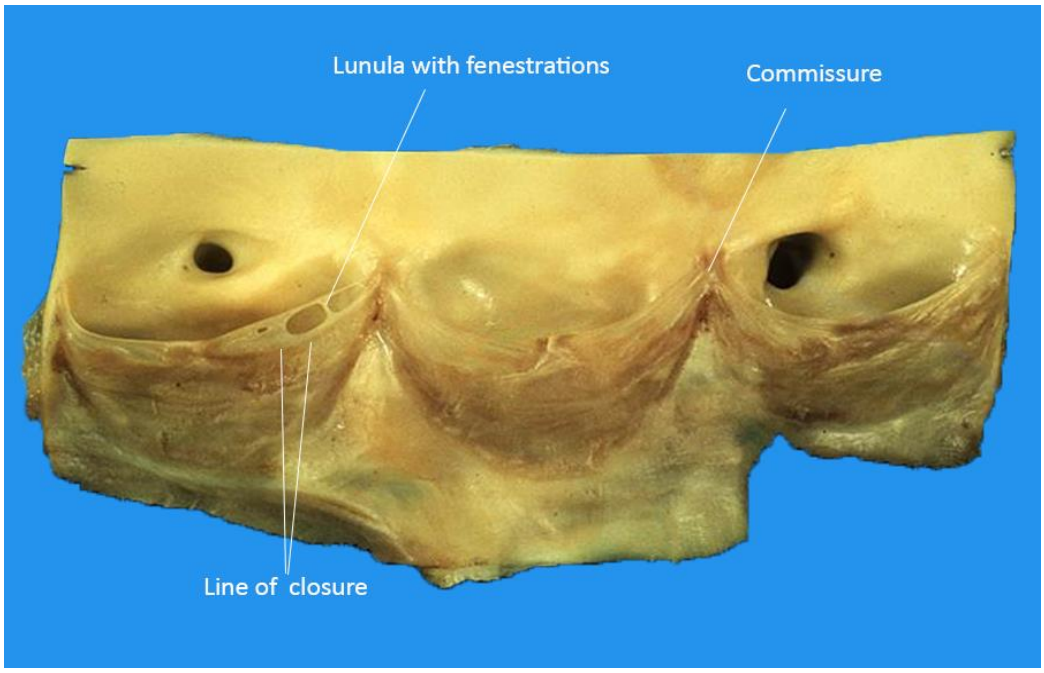

Figure 1.8: Pathological human specimen of a trileaflet AV showing the commissure between the non-coronary and right coronary cusps [20].

Sinuses of Valsalva

The three bulges of the aortic wall are named the SOV, after the Italian anatomist Antonio Valsalva. Two of the three sinuses host the origin of the coronary arteries, and the sinuses are named accordingly as the left, right and non-coronary sinus. The limits of the sinuses are the attachments of the valve leaflets (proximally) and the STJ (distally). The bulges act as vortices and lead to stress reduction on the aortic leaflets and support coronary flow [Figure 1.9] [21].

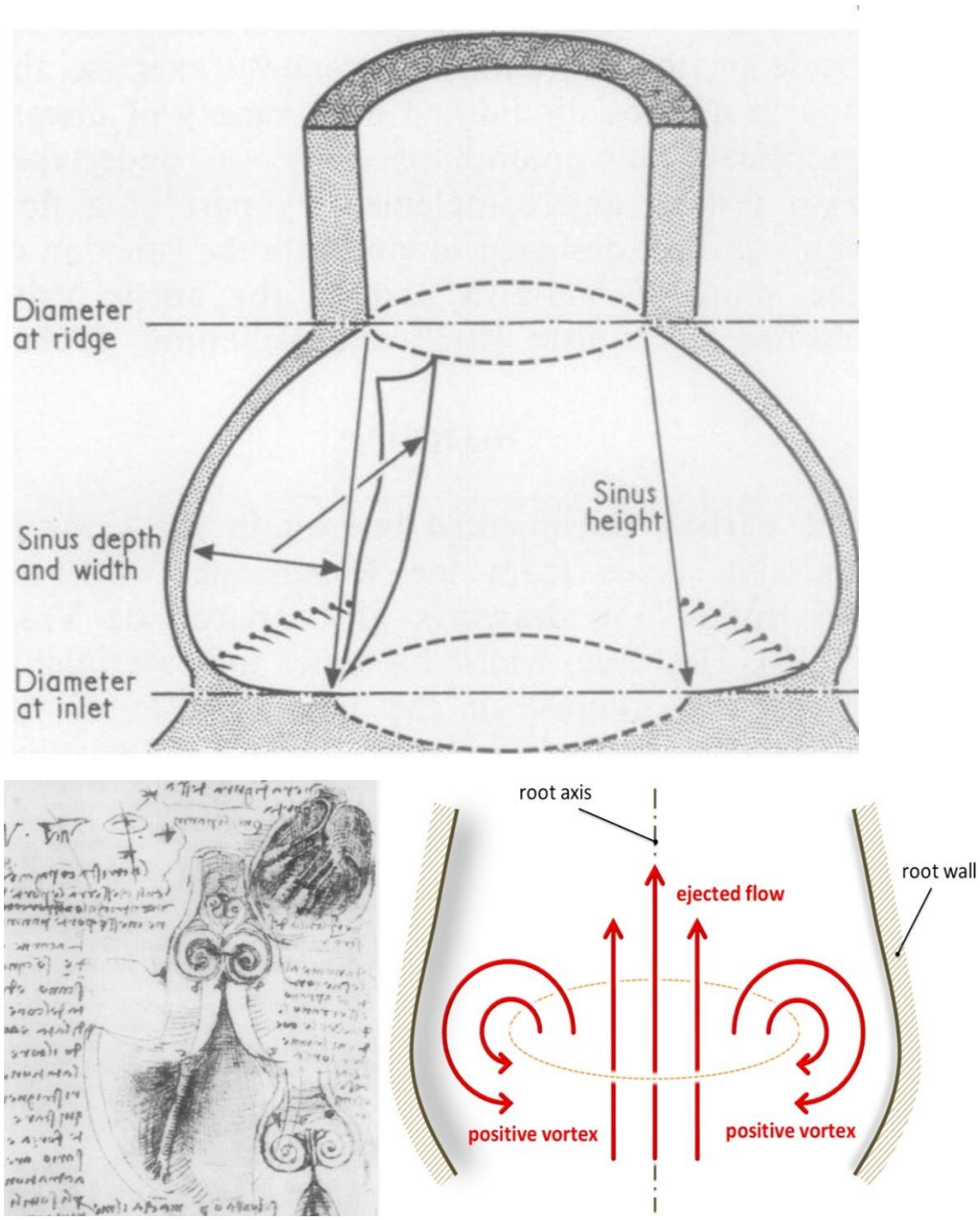

Figure 1.9: Diagrammatic representations of the SOV and the associated effects on blood flow. Top image – Diagram of the aortic root showing the shape of the sinuses, in particular their depth and width in relation to the remaining root [21]. Bottom left image – Leonardo Da Vinci early depiction of flow visualisation in the heart [21] and Bottom right image – Diagram describing a positive vortex ring formed during cardiac ejection showing the opposite direction of flow at the arterial wall [22].

Interleaflet triangles

Under each commissure lies one of the three interleaflet triangles (Figure 1.10). Haemodynamically, they are extensions of the ventricular outflow tract and reach the level of the STJ in the area of the commissures.

The triangle between the right- and non-coronary sinuses faces the right atrium. It is in direct continuity with the membranous septum proximally which contains the His bundle. Under the left and non-coronary triangle, the aorto-mitral curtain leads to the anterior mitral valve leaflet [3].

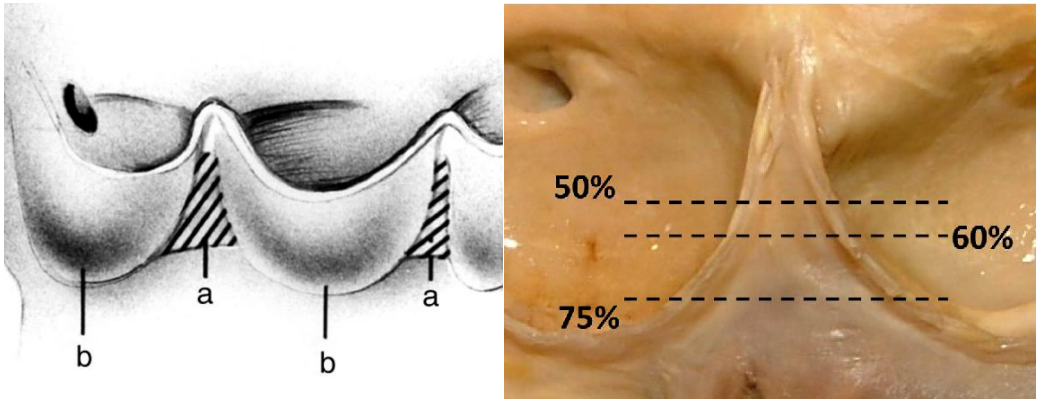

Figure 1.10: The interleaflet triangle anatomy. Left image - Diagram of the aortic root opened longitudinally through the left coronary sinus, demonstrating the interleaflet triangles (a) and the valve leaflets (b) [23] Right image - Pathological human specimen of the interleaflet triangles in between each coronary sinus with lines referencing landmarks for AV repair [24].

Sinotubular junction

The distal part of the sinuses toward the ascending aorta together with the commissures form a tubular structure called the STJ, which separates the aortic root from the ascending aorta (Figure 1.11) [25]. In some cases, dilatation of the STJ is the cause of central aortic insufficiency and replacement of the ascending aorta with a short tubular graft can restore valve competence.

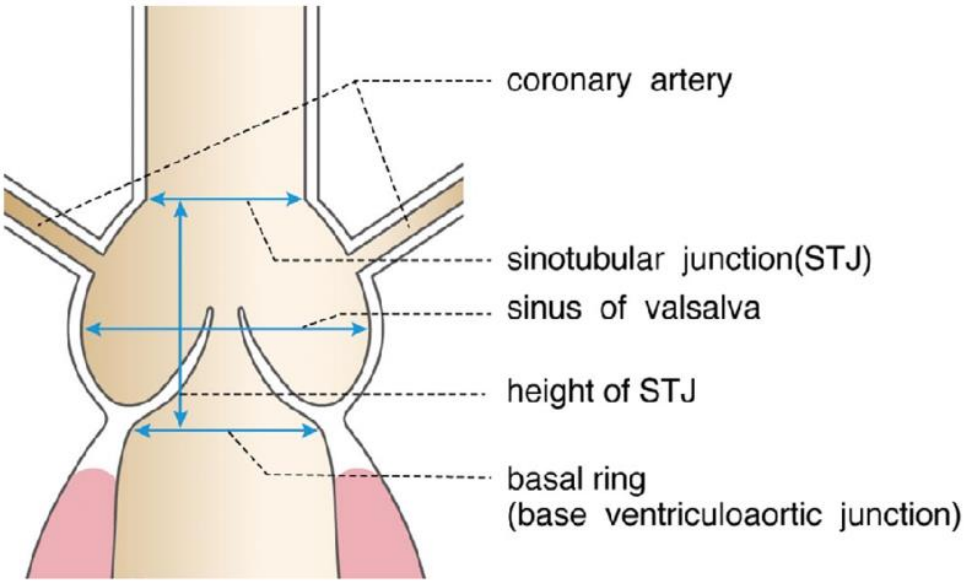

Figure 1.11: Cross sectional view of the aortic root showing the location, diameter, and height of the STJ [25].

Annulus

Although the word annulus implies a circular structure, no distinct histological entity or anatomical boundary fits this description. The circumference defined by the nadirs of the semi-lunar leaflet attachments is difficult to define as the annulus, because there is no real, anatomically, or histologically distinct, circular structure. The term 'ventriculo-arterial junction', as a definition of the "annulus", is rather ambiguous as the 'anatomical ventriculo-arterial junction' represents the junction between the left ventricular myocardium and the arterial structure of the aorta. On the contrary, the 'haemodynamic ventriculo-arterial junction' is represented by the coronet shaped leaflet insertion and defines the separation level of ventricular and arterial haemodynamics. From a strictly anatomic point of view, the 'anatomic/histologic ventriculo-arterial' as well as the 'haemodynamic ventriculo-arterial' junction lie somewhat more distally to the 'annulus' [4, 26, 27] and defines the area of interest less precisely (Figure 1.12).

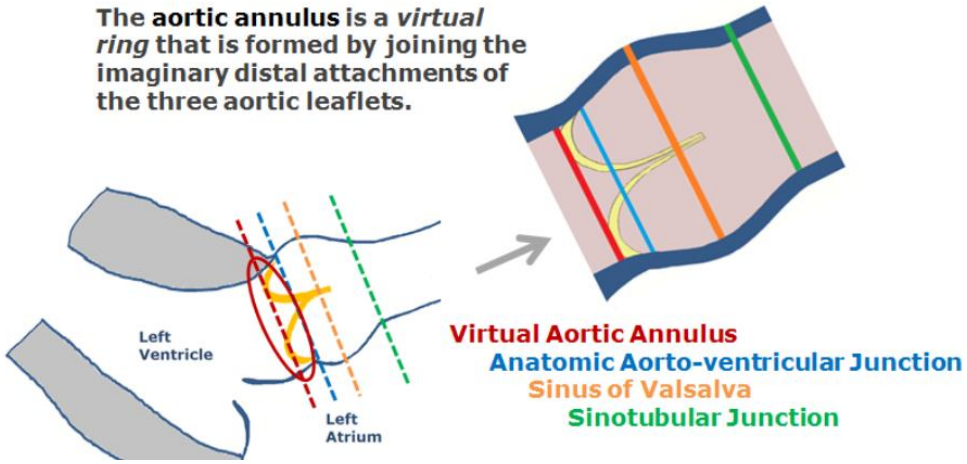

Figure 1.12: Diagram showing the aortic annulus anatomical landmarks, colour coded to their location [26].

Despite the absence of any anatomically or histologically distinct circular structure the popularity of the term 'annulus' probably stems from the fact that this is the area of the smallest diameter in the blood path between the left ventricle and the aorta and determines the fitting position of prosthetic valve sizers and, therefore, the size of the prosthetic valve to be implanted. In addition to this, the use of this definition gives a good impression of the operative technique in use, such as the positioning of the prostheses 'supra' or 'intra-annular', as this is the level measured by echocardiography and is the area which defines the size of the prosthesis to be implanted during aortic valve replacement procedures. However, prosthetic valves are inserted somewhat more proximally, more towards the level of the anatomic ventriculo-arterial junction, due to the placement of the sutures predominantly through the scalloped attachment of the excised leaflets, from the nadir of the sinus to midway up the commissures [3, 28, 29]. To avoid any misunderstanding due to the numerous definitions and terms employed; proposed terms for the "annulus" have been to describe the virtual, circular ring defined by the nadirs of the semi-lunar leaflet attachments [3].

1.3 Histology

1.3.1 Macroscopic structure

To understand the macroscopic details, the AV must be seen in context with its structural unit, the aortic root. It is the connecting part between the left ventricle and the ascending aorta and is found in a position wedged between the left and right atrioventricular annuli and the bulging thick left ventricular myocardium. The aortic root is vital in its support for the AV and forms the anatomic boundary between the left ventricle and the aorta.

1.3.1.1 Aortic root and aortic valve

As shown in detail in the anatomical descriptions, the AV is a component of the aortic root. The crown shape annulus, the three SOV and interleaflet triangles, as well as the STJ, commissures and the AV leaflets interact with each other and form an integrated root structure . This well-coordinated dynamic behaviour has been shown to be of importance for specific flow characteristics, for coronary perfusion and left ventricular function [27].

Annulus

Macroscopically, the annulus is a well-defined fibrous structure that is firmly attached to the media of the aortic sinuses distally, while proximally it is attached to the muscular and the membranous septa anteriorly, the fibrous triangles laterally and the subaortic curtain posteriorly. The three upper parts of the annulus are called commissures [27]. The virtual annulus is obtained by joining the lower points of the three leaflets, a circumferential ring that is not anatomically or histologically defined. The insertion of the leaflets on the aortic wall takes the form of three prolonged coronets with the lowest part (called nadir) lying slightly below the ventricular–arterial junction and the highest point joining the STJ [30, 31]).

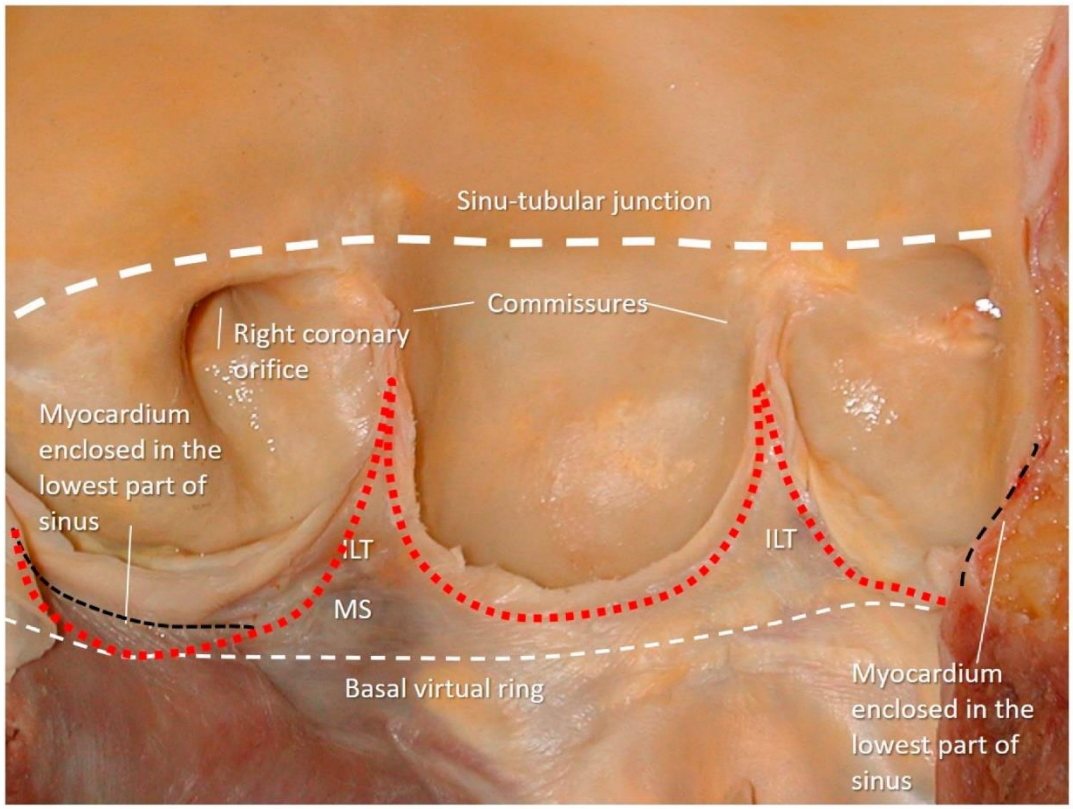

Figure 1.14: Anatomical specimen components of the aortic root. The crown-shaped annulus is depicted by the red-dotted line, and the virtual basal ring by the white thin dotted line (MS – membranous septum, ILT = interleaflet triangle) [30].

Commissures

The commissures are of fibrous structure and suspend the valve leaflets. They are located above three triangular areas called interleaflet triangles.

Interleaflet triangles

The interleaflet triangles are extensions of the ventricular outflow tract, consisting of thinned aortic wall. This interleaflet triangle is in fibrous continuity proximally with the membranous septum (Figure 1.15), which itself is in fibrous continuity with the right fibrous trigone (yellow diamond), with these two latter structures together creating the central fibrous body.

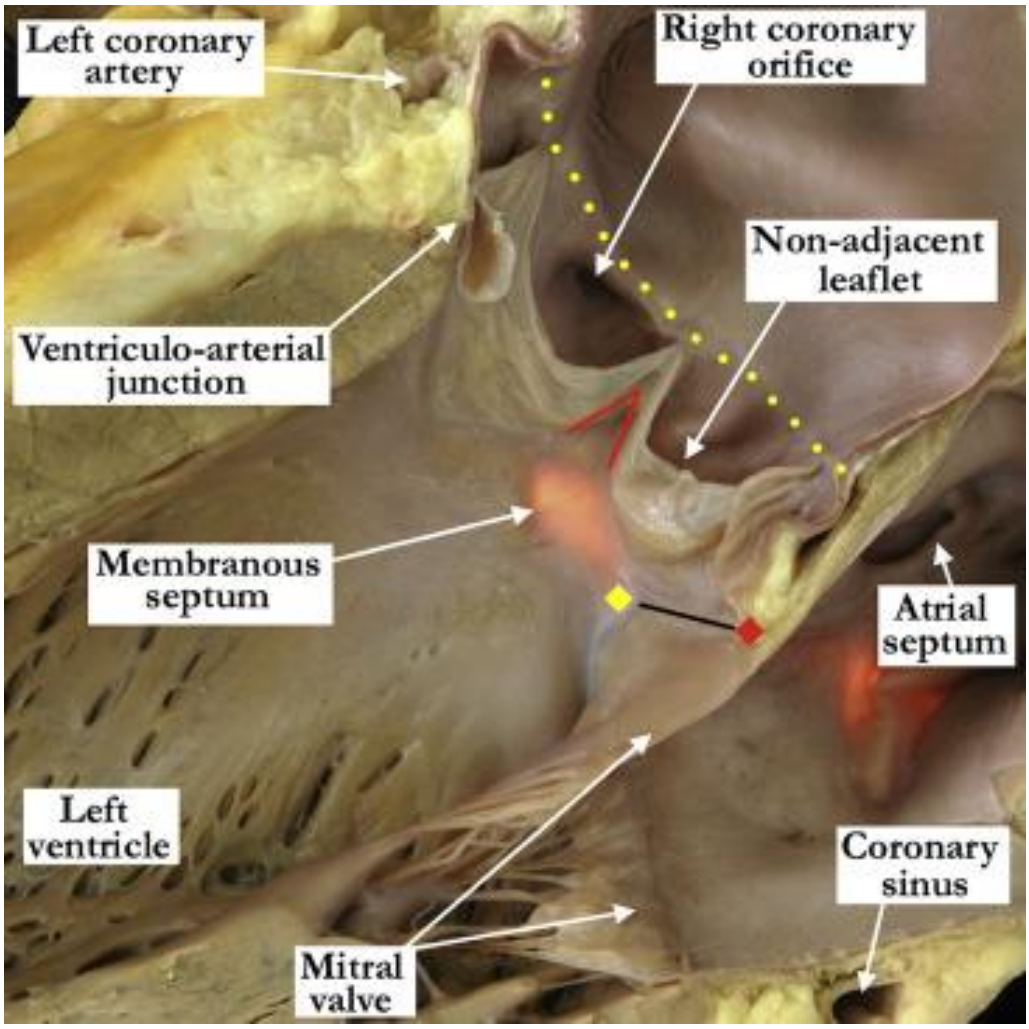

Figure 1.15: Left atrium, left ventricle and aortic root are opened in a sagittal plane demonstrating the labelled anatomy. The membranous septum is transilluminated to demonstrate its continuity with the interleaflet triangle [31].

The area of fibrous continuity between the anterior leaflet of the mitral valve and the entirety of the noncoronary leaflet and portion of the transected left coronary leaflet of the AV is demonstrated (black line), resting between the right (yellow diamond) and left fibrous trigones (red diamond). This demonstrates the intricate macroscopic relationship between the aortic and mitral valve (Figure 1.16).

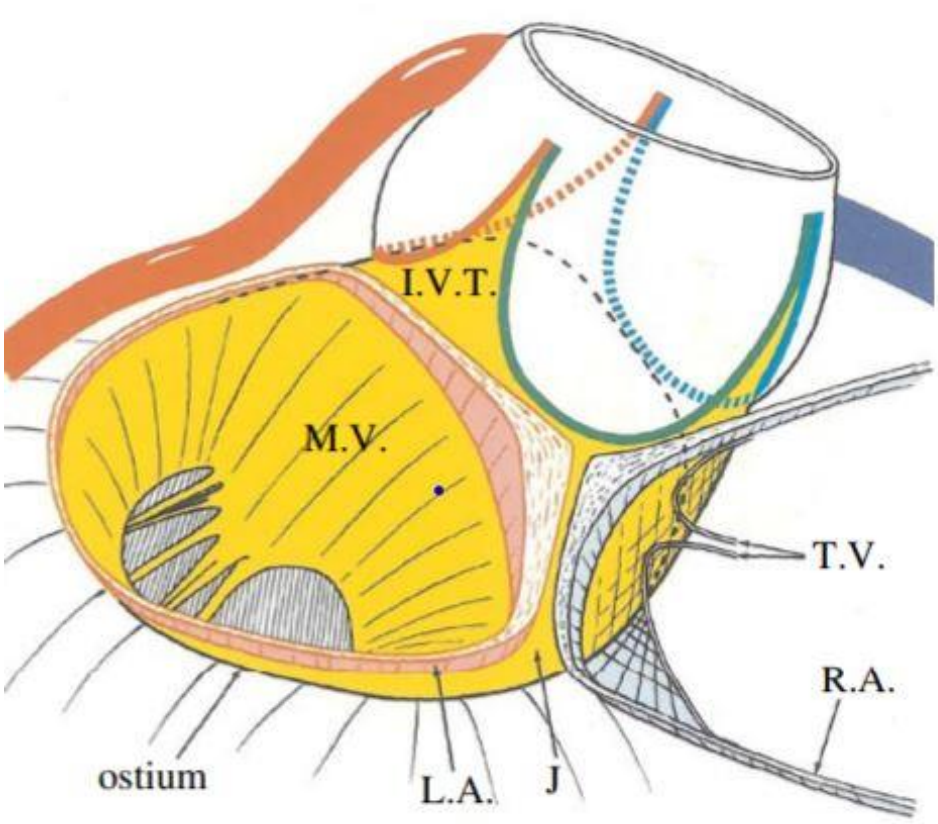

Figure 1.16: After removal of the right atrium, this diagram illustrates the continuity of the AV and mitral valve structures. Note the large intervalvular trigone (IVT). MV – mitral valve, TV – tricuspid valve, LA – left atrium, J – junction between atrioventricular valves [27].

Sinuses of Valsalva

As previously described, the three sinus bulges are confined proximally by the attachments of the valve leaflets and distally by the STJ, giving rise to the coronary arteries at specific points. They contain some ventricular musculature at their bases. The sinus wall itself is mainly made up of a thinner aortic wall structure, and the sinus wall itself is predominantly aortic wall tissue, although it is thinner than the native aorta.

Sinotubular junction

The superior border of the sinuses is the STJ (also known as the supra-aortic ridge). On the outside, the STJ is where the tubular portion of the aorta joins onto the sinusal portion. Inside, there is usually a slightly raised ridge of thickened aortic wall. But the STJ is not perfectly circular. It takes on the contour of the three sinuses, giving it a mildly trefoil or scalloped outline [31]

Valve leaflets

The AV leaflets consist of four components: the hinge, the belly, the coapting surface and the lannula with the nodule of Arantius.

The noduli of Arantii are located at the midpoint of the free edge of the coapting surface. On either side of this nodule is a thin crescent-shaped portion called the 'lannula'. This lannula consists of a thin margin at its free end and continues in the coaptation region where the three leaflets meet each other and ensure complete valve closure. The lannula are attached to the wall of the aortic root in the area of the commissures. The main part of each leaflet is called the belly, where the leaflets appear to be almost transparent. Macroscopically, the specific arrangement of collagen structures of each leaflet can be identified. The component where the leaflets are attached to the annulus in a semilunar shape is called the hinge area, where the leaflet attachment crosses the ring-like junction of the aortic wall and the ventricular mass. The thick collagenous bundles of the leaflets are hinged to the annulus allowing for transmission of stress on the leaflets to the aortic wall. In terms of leaflet sizes, the non-coronary leaflet tends to be the largest, followed by the left coronary leaflet and the right coronary leaflet [27].

1.3.1.2 Ascending Aorta

The ascending aorta is a type of elastic artery and the largest vessel in the body. The arterial walls of the circulatory system generally are composed of three layers, but the layers vary depending on the type of artery. There are three layers to the aortic wall, the tunica intima, tunica media, and tunica adventitia. Elastic arteries contain much more elastic tissue in the tunica media than muscular arteries. This feature of the elastic arteries allows them to maintain a relatively constant pressure gradient despite the constant pumping action of the heart. [32, 33].

The adventitia is the thin outermost collagenous layer that contains the vasa vasorum and nerves, and despite its thin structure, collagen gives it great tensile strength. The media is the thick middle layer that normally accounts for up 80% of the aortic wall thickness and consists of elastic tissue intertwined with muscle fibres. The aortic intima is the thin inner wall layer, characterized by basement membrane lined with endothelium that is in direct contact with the blood. The intima is most prone to injury due to its delicate structure.

Ascending aorta morphology has been well described in animal studies. The tunica media of the wall of the ascending aorta is described as containing elastic fibres interspersed with an abundance of vasa vasorum, with entrances to the vasa vasorum able to be seen from the lumen of the aorta on examination. A complex network of vessels exists inside the wall that seems to terminate in a profusion of veins near the adventitial surface [Figure 1.17] [34].

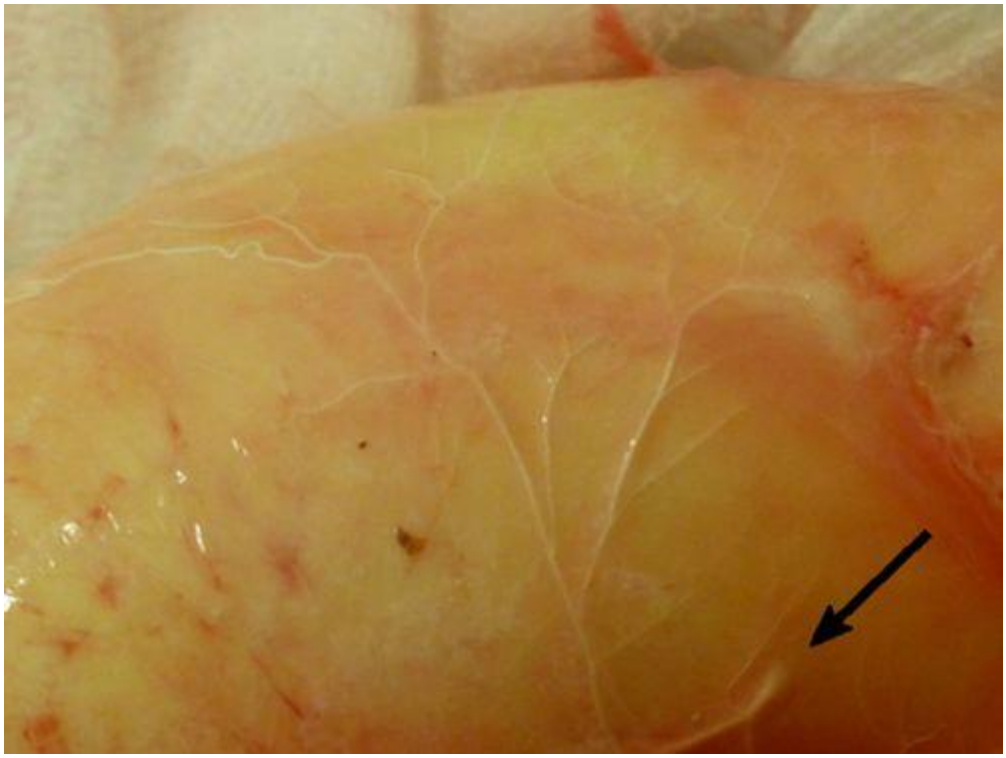

Figure 1.17: Photograph of the outer surface of the ascending aorta after infusion of barium sulphate solution (white) into the lumen of the vessel under arterial pressure, showing the emergence of a major vessel (arrow), which subsequently branches out into an arterial tree in the adventitia of the aorta [34 was 29].

1.3.2 Microscopic structure

The diverse smooth muscle cell origin of the thoracic arteries and its implications to disease development have been long known and the histological structures of the AV have been well described [27]. The root is populated by a smooth muscle subtype that originates from the lateral plate mesoderm, whereas the subtype of the ascending aorta is neural crest derived [35, 36]. This embryological difference influences a histological shift from the muscular ventricle to the primary elastic aorta in the root and ascending aorta. The changes in the valve cusps and leaflets that occur during the cardiac cycle are a result of a complex internal microarchitecture within the aortic valve leaflets, but also within the aortic root microstructure itself.

1.3.2.1 Aortic root and aortic valve

Annulus

The annulus is a dense collagen mesh with elastin and collagenous fibrils, with radially orientated collagen fibres in the intermediate layer of the commissures anchored in the medial layer. Passing through this structure is the non-coronary sinus, where no myocardial muscle supports the sinus, giving the appearance of a cartilaginous structure. The ventricular and arterial layer divide apart, and the intermediate collagenous layer shows a cuneiform structure. The ventricular layer continues as the endocardial layer, whereas the arterial layer continues into the sinus wall. Small vessels and neuronal structures are in the connective tissue layer [27].

Commissures

The force on the closed valve is transmitted to the annulus primarily by a system of collagen fibres that originate at the commissure level. The collagen fibres of the intermediate layer are orientated radially in the area of the commissures. The collagen fibres do not only infiltrate the intima layer of the aortic root; but they radiate and anchor themselves within the medial layer [27].

Interleaflet triangle

The three triangles are not bounded by ventricular musculature, but by a thinned fibrous wall of the aorta between the expanded sinuses. It is histologically fibrous and equivalent to the mitral valve leaflet structure. The triangle between the non-coronary and the right-coronary aortic sinus is incorporated within the membranous part of the septum and is also made of fibrous tissue, while the triangle between the right-coronary and left-coronary sinus in the area of the subpulmonary infundibulum is supported by muscular tissue and is only fibrous at its apex.

Sinuses of Valsalva

Arteries are connected to the heart with so-called arterial fibre-rings, which are described as tendon-like and have poorly defined boundaries. The sinuses are therefore arranged with very different components. The largest part of the sinuses, however, is structured like the three layers of the aortic wall (intima, media and externa or adventitial layers). The inner layer of the intima is composed of endothelial cells and subendothelial connective tissue arranged in the direction of the vessel. This layer is divided from the intima by the membrana elastica interna. The media is composed of circular arranged structures: smooth muscle cells, elastic fibres, collagen fibres type II and III and proteoglycans. The adventitia is the external layer and is separated from the intima by the membrana elastica externa. Like the intima, the elements of the externa are arranged in a longitudinal fashion and composed of collagen fibres of type I. Although the wall of the sinuses is principally arranged in this manner, the thickness of its wall is significantly thinner compared with the ascending aorta

The STJ is similar in structure but is described as having a thicker wall [27].

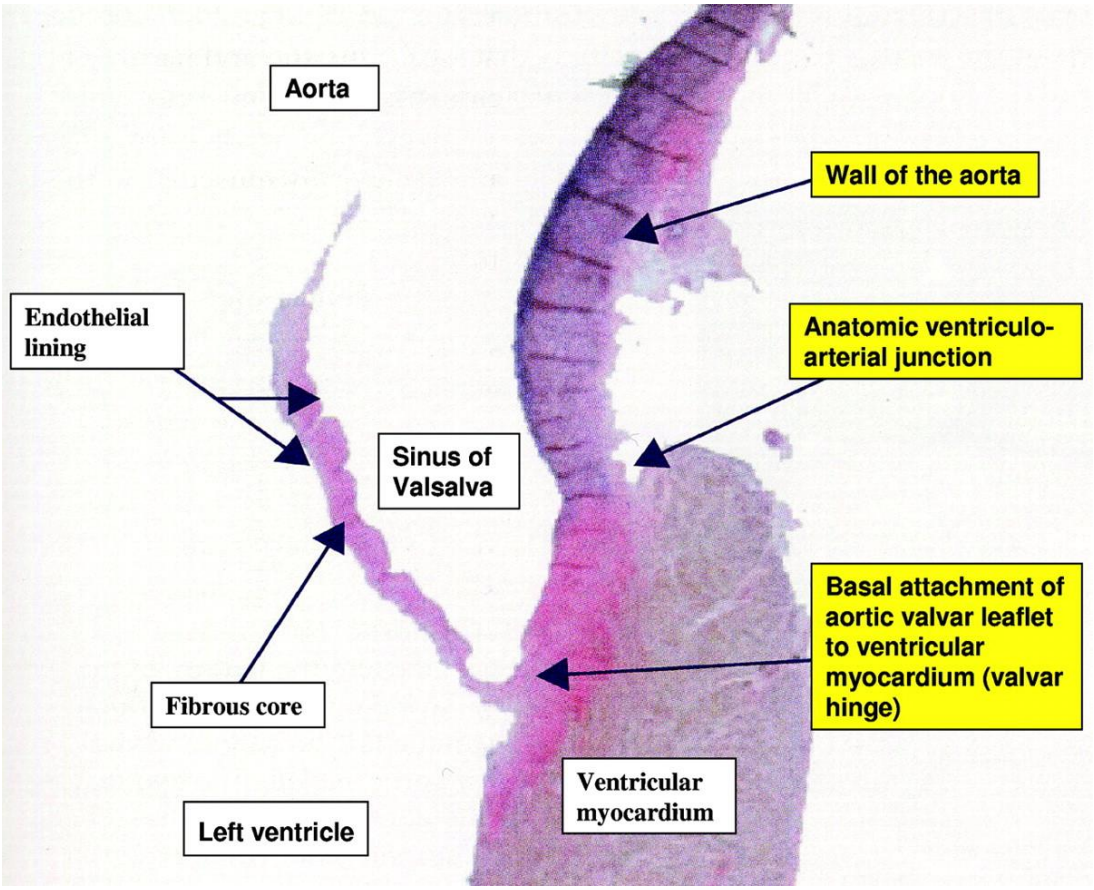

Figure 1.18: Histology of the aorto-valvular complex. The basal attachment of the aortic valvar leaflets to the ventricular myocardium is proximal relative to the anatomic junction. Image adapted from [4].

Sinotubular junction

The STJ microscopic structure shows the same principal arrangement of tissue elements compared with the sinuses and the ascending aorta. The diameter of the wall is thicker than the diameter of the sinus wall, and this area defines the ridge as the upper part of the aortic root.

Leaflets

The AV leaflets are covered by a continuous layer of endothelial cells with a smooth surface on the ventricular side and ridges on the arterial side, joined together by junctions [Figure 1.18]. The arrangement of the endothelial cells is across, not in line with the direction of flow. Between the ventricular and aortic surfaces, there are up to five layers of connective tissue: lamina ventricularis, radialis, spongiosa, fibrosa and arterialis (Figure 1.19) [35]. Within the connective tissue, the elastic

and collagen fibres show a preferential arrangement and orientation. The arterial layer contains coarse bundles of circumferential collagen fibres, which form the macroscopical folds parallel to the free edge of the leaflets. It is this arrangement of fibres that transfers the load of the leaflets to the wall of the aortic root. Between the extracellular components reside interstitial cells. Initially described as smooth muscle cells these cells show characteristics of fibroblasts and smooth muscle cells, and have been therefore designated as myofibroblasts.

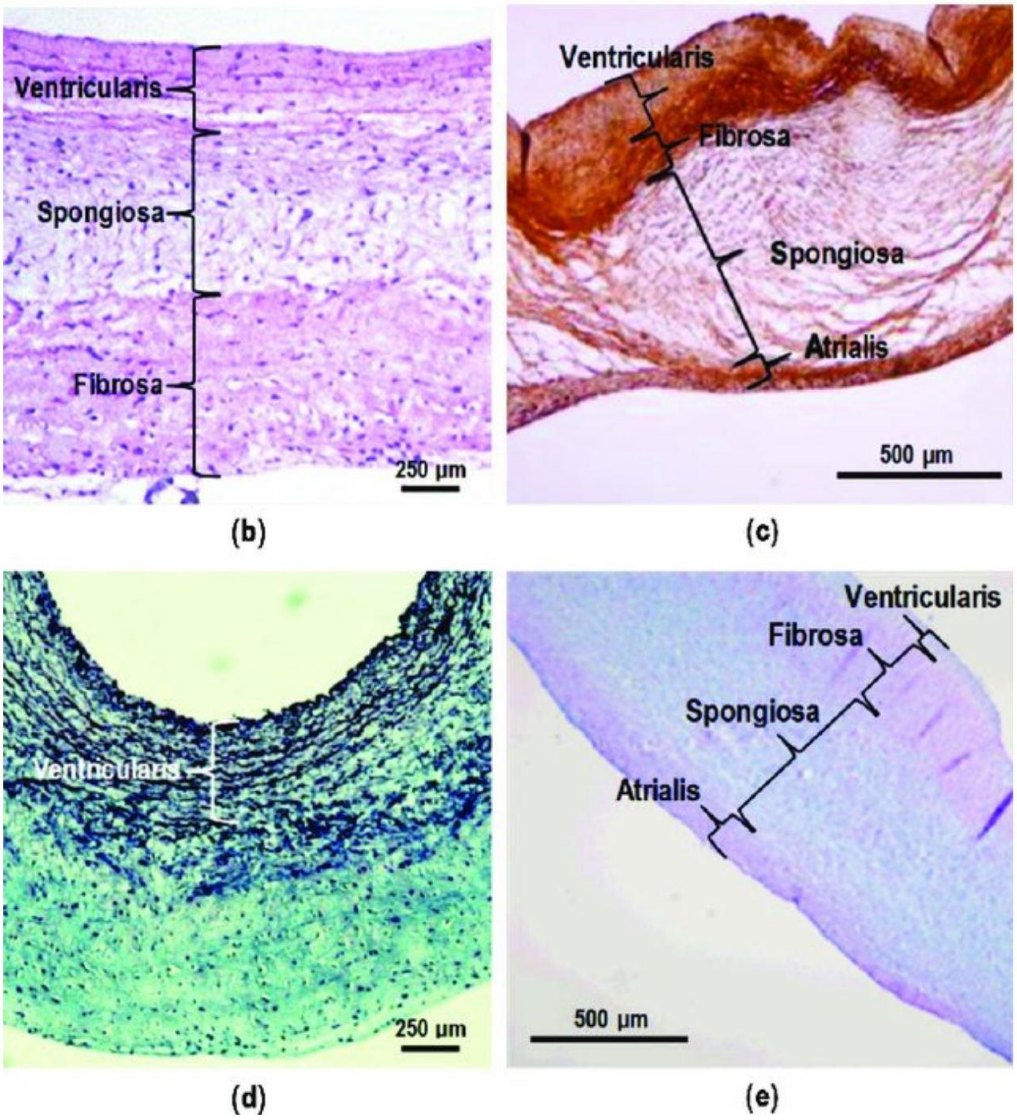

Figure 1.19: (a) Trilaminar leaflet structure of semilunar valves, showing the fibrosa, spongiosa and ventricularis layers, together with their major constituents. (b) H&E histological staining of the AV leaflet (radial direction). ECM proteins were stained pink/light purple; cells were stained deep/purple. (c) Immunohistochemical staining against collagen I. Collagen I was stained brown. (d) Miller's elastic histological staining of the AV leaflet (radial direction). Elastic fibres were stained deep blue/black. (e) Alcian blue/PAS histological staining showing the ventricularis, fibrosa,

spongiosa and atrialis layers; dark blue: cell nuclei; blue: acid mucosubstances (GAGs) and proteoglycans; magenta: neutral polysaccharides [35].

1.3.2.2 Ascending aorta

The microscopic structure of the human adult aortic wall comprises the three layers as described. Intima is composed of a monolayer of endothelial cells supported by a special type of connective tissue (subintima), with a basement membrane between the two types of tissues (Figure 1.20) [36].

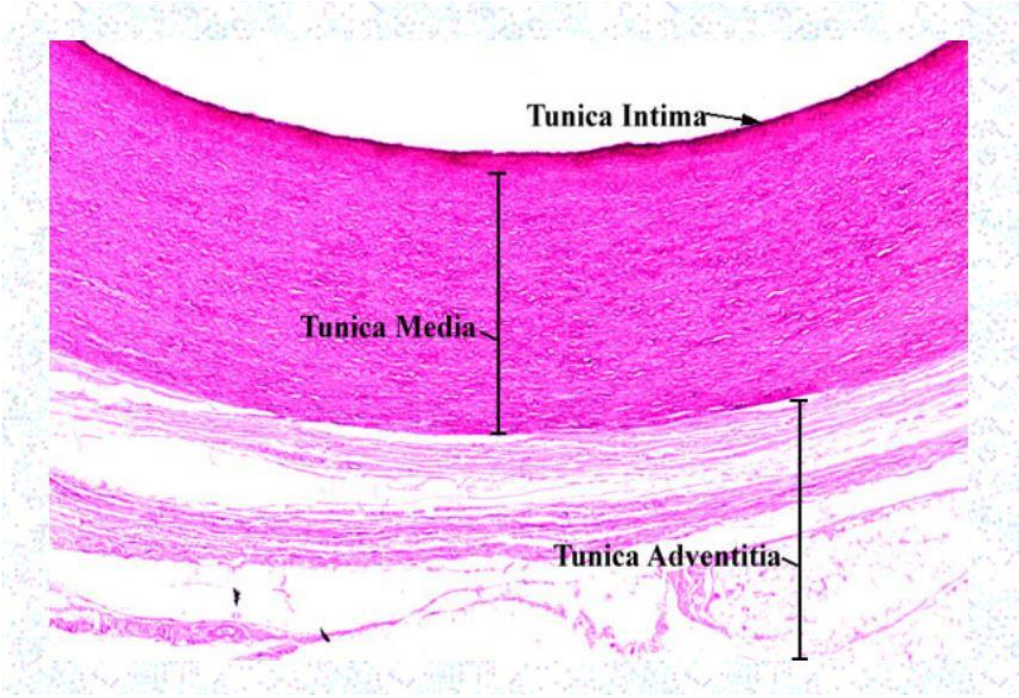

Figure 1.20: Microscopic layers in the human aorta as labelled. The very thin inner layer (Intima), the thicker muscular middle layer (Media) and less densely packed outer layer (Adventitia) [37].

The endothelium is continuous with endocardium and represents the interface between the vascular wall and blood [36]. It is actively involved in the production and reaction to inflammation mediators, and a wide range of cytokines [36]. The basement membrane is composed of type IV collagen and laminin. The subendothelial layer contains collagen type I and II, elastic fibres, abundant extracellular matrix rich in proteoglycans, phenotypic myocytes, myointimal cells, and macrophages.

An internal membrane composed of condensed elastic fibres determine the boundaries of the intima and media. Media is composed of concentric elastic lamellae with smooth muscle cells, multiple types of collagens, and proteoglycans [38], and external elastic lamina [39]. Media occupies approximately 80% of the wall thickness and contains up to 70% elastic lamellae.

Adventitia is composed of connective tissue type I collagen fibres, elastin, and fibroblasts [39], with associated vasa vasorum and nervi vasorum. The aorta is an elastic artery of which the main structural components are elastin and collagen fibres, smooth muscle cells, and a proteoglycan-rich ground substance (Figure 1.21). Over time, the intimal layer gradually expands and is composed of extracellular matrix proteins (mainly collagen and mucopolysaccharides, as well as sparse mesenchymal cells). The media constitutes the largest component of the artery, and is composed of concentrically arranged elastic laminae that enclose smooth muscle cells, collagen fibres, and large amounts of proteoglycans. In contrast to muscular arteries, the aorta contains no prominent internal elastic lamina, nor does it have a distinctive external elastic lamina. The adventitia is composed of loosely arranged connective tissue, vasa vasorum, lymphatic vessels and low numbers of perivascular leukocytes. The vasa vasorum normally extends into the outer third of the media. It must be noted that the "normal" aorta at older ages displays increasing degenerative changes of all structural components, as described later (age-related changes), related to longstanding (many decades) "wear and tear" [39].

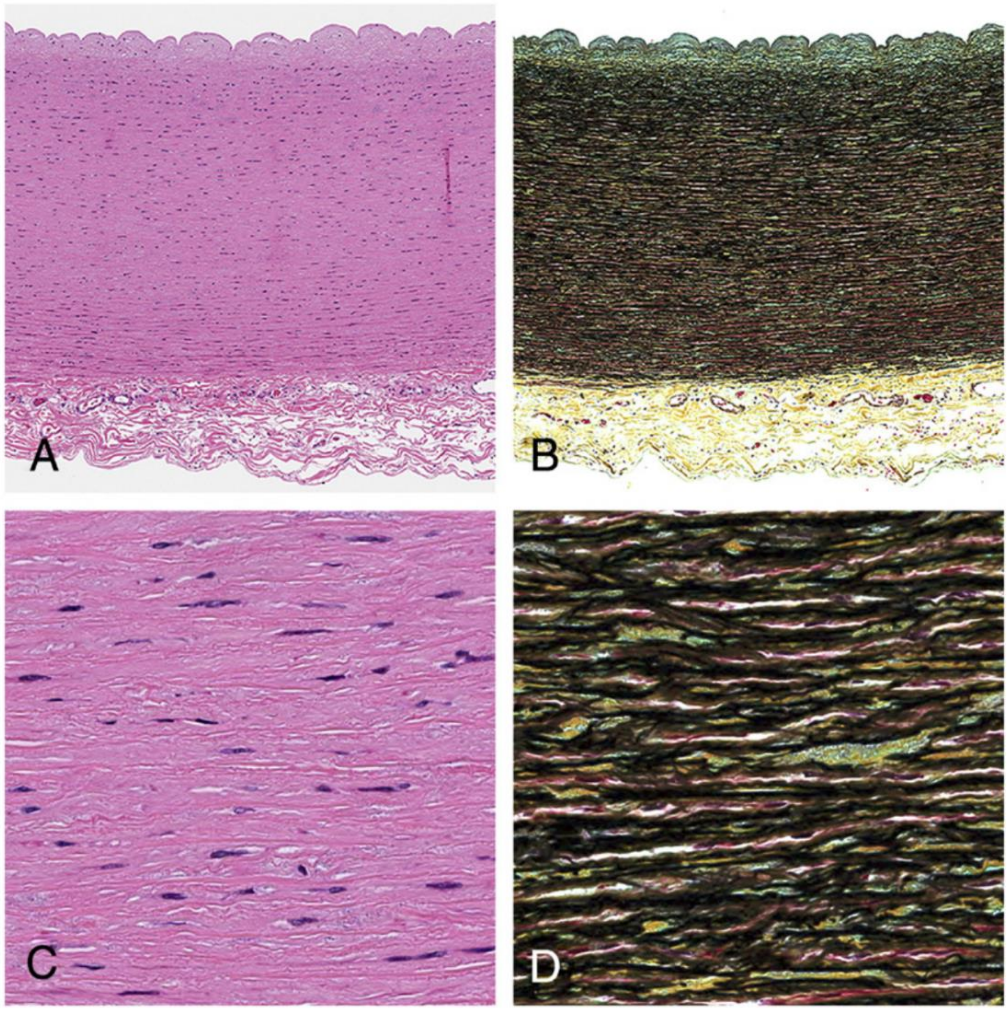

Figure 1.21: Normal aorta in a young adult. (A) Transverse section demonstrating all three aorta layers: intima at the luminal surface (top), media, and adventitia (50x, H&E). (B) On this stain highlighting elastic fibres, the intima is a distinctly paler layer than the media. The media consists of multiple lamellar units highlighted by the black lines of elastic laminae. There is an abrupt change at the boundary of the media and adventitia. (50x, Movat's pentachrome). (C) At higher magnification, the media shows distinct lamellar units with slightly more eosinophilic and refringent elastic laminae. The majority of the smooth muscle cell nuclei are seen in longitudinal orientation as this is a section perpendicular to the longitudinal axis of the aorta (500x, H&E). (D) The lamellar units in close. (500x, Movat's pentachrome) [39].

1.3.2.3 Collagen content of the normal aorta

Collagen is thought to be one of the most important components of the aortic wall. The amount of collagen and the collagen type ratios in the aortic wall can change with ageing, influence of sex hormones and pathology (aneurysms, hypertension) [41].

The aortic media contains elastin, collagen, smooth muscle cells, and a nonfibrous matrix. Elastin and collagen account for 50% of the dry weight. The modulus of elasticity of collagen is approximately 400 times greater than that of elastin. Light and electron microscopic studies of the aortic wall indicate that medial structural components are arranged in an orderly fashion; concentric fibrillar elastin lamellae are connected by an intricate network of elastin fibrils with interspersed collagen fibres and smooth muscle cells [42]. The orientation of and close interconnection between the elastic and collagen fibrils, elastic laminae and smooth muscle cells together constitute a continuous fibrous helix. The helix has a small pitch so that within the media it is almost circumferentially oriented. This structured arrangement gives the media an ability to resist high loads in the circumferential direction [Figure 1.22] [45].

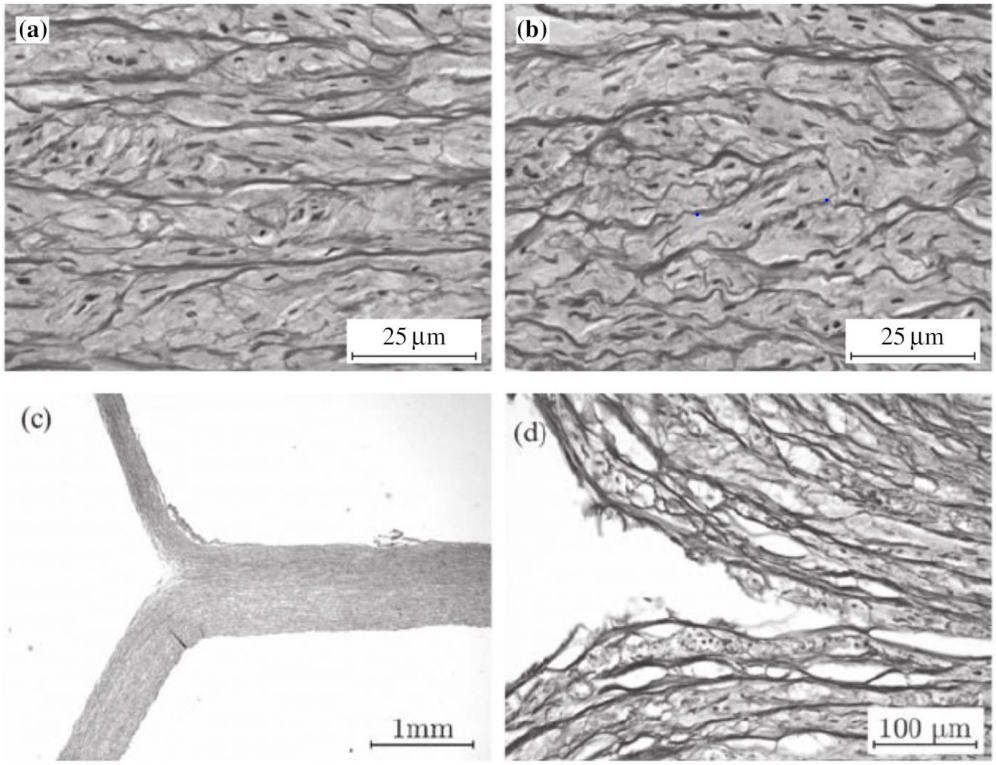

Figure 1.22: Histological images of a representative media from a human aorta: (a) stretched and (b) unstretched samples demonstrating the microstructure of the media (original magnification 800×); (c) media dissected during peeling in the axial direction (original magnification 20×); (d) magnification of the dissection tip showing pronounced fibre bridging and a cohesive zone (original magnification 400×). Elastica van Gieson staining, 4 micro/metre thick sections [45].

The intima is a single layer of endothelial cells lining the arterial wall, resting on a thin basal membrane. With time, intimal cells (mainly myofibroblasts) proliferate concentrically and lead to an increase of extracellular matrix containing mainly collagen fibres within, and dispersed smooth muscle cells throughout the layer. The orientation of the distinct families of collagen fibres is dispersed. The adventitia is surrounded continuously by loose perivascular tissue and consists

mainly of fibroblasts and fibrocytes, histological ground-matrix and collagen fibres organised in thick bundles. Polarised light microscopy of the structure of the adventitia has shown that the collagen forms two helically arranged families of fibres [Figure 1.23] [43].

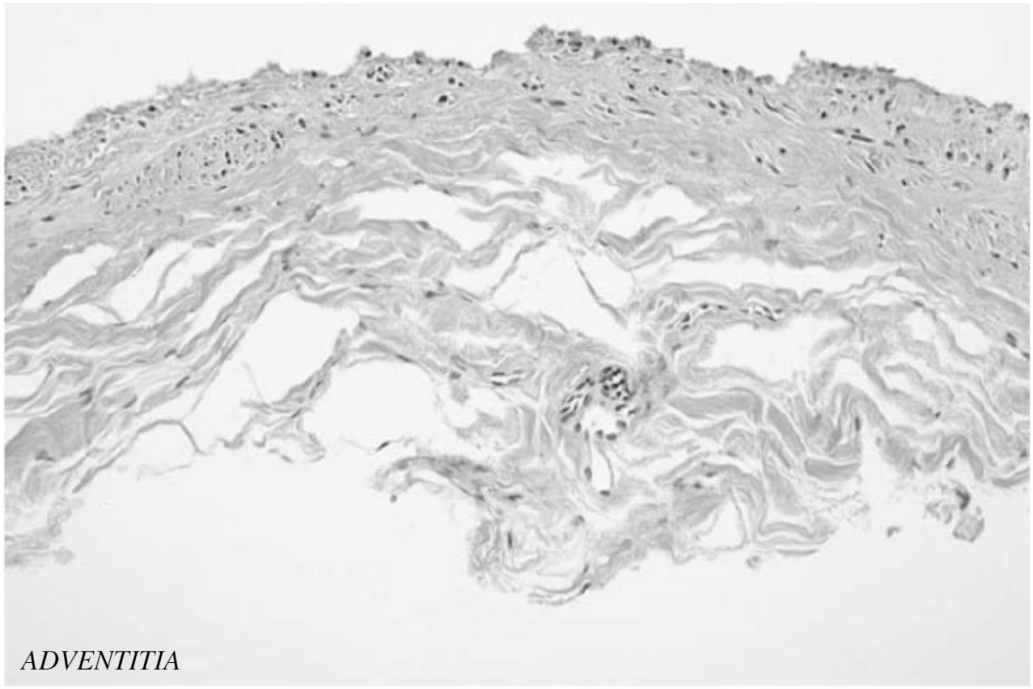

Figure 1.23: Photomicrograph of a 3 micro/metre thick adventitia sample, obtained from an aged human coronary artery and stained with Hematoxylin and Eosin. Note the tendency to separate because of loose collagen fibres in the outer part of the adventitia. Original magnification 200× [43].

Studies examining collagen in the human aorta across various ages showed a significant positive correlation for collagen content with age and a significant increase in the number of hydroxyproline residues (indicative of amount of collagen) after age 50.

1.3.2.4 Elastin content in the normal aorta

Elastin is an extracellular matrix (ECM) protein with a unique biochemical structure that provides entropic elasticity, allowing the large arteries to reversibly expand and relax with every cardiac cycle. Insufficiency, disorganisation, improper assembly, fragmentation, and biochemical modifications of elastic fibres change the passive mechanical behaviour of the large arteries and affect cardiovascular mechanics. Both genetic and acquired cardiovascular diseases are associated with elastin and elastic fibre defects and the resulting changes in arterial mechanics [44].

The middle and largest layer of the aorta, the media, is further divided into sheets of elastic fibres or elastic laminae. These laminae are separated by a region composed of smooth muscle cells, thin

elastic fibres, collagen (mostly types I and III), and proteoglycans. An internal elastic lamina separates the media from the intima. Elastic fibres and collagen interconnect the elastic laminae forming a continuous network with a three-dimensional helical structure in which the fibres are oriented circumferentially [Figure 1.24].

The amount of elastin is highest in the large, elastic arteries closest to the heart and decreases as one moves distally in the cardiovascular system [44]. Elastic fibres provide reversible elasticity to the large, elastic arteries, allowing the aorta to deform elastically under an applied haemodynamic load, with no permanent deformation and no energy dissipation when the load is removed [44].

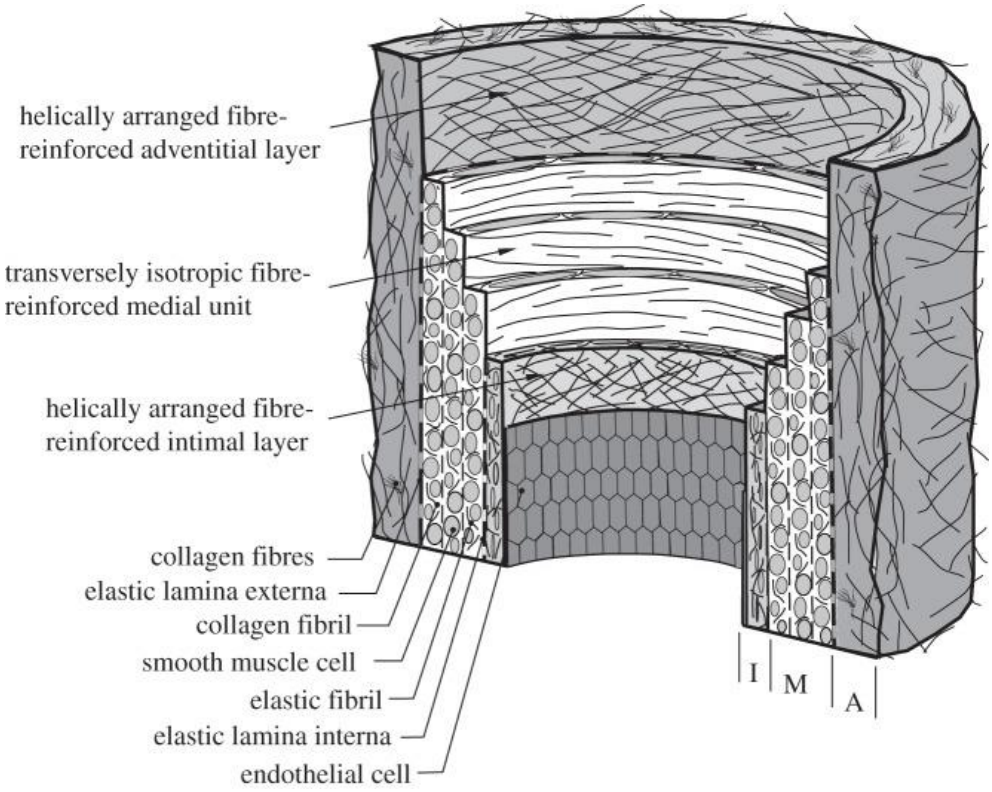

Figure 1.24: The generalised internal structure of the human artery with layers labelled as follows: (intima (I)), middle layer (media (M)) and outer layer (adventitia (A)). The intima is composed mainly of a single layer of endothelial cells, a thin basal membrane and a subendothelial layer of collagen fibrils. The media is composed of smooth muscle cells, a network of elastic and collagen fibrils, and elastic laminae which separate M into transversely isotropic fibre-reinforced units. The adventitia is the outermost layer surrounded by loose connective tissue [45].

1.4 Pathology

1.4.1 Pathology of the thoracic aorta and aortic root

Thoracic aortic aneurysms (TAAs) are typically described according to their location. Approximately 60% involve the ascending aorta and 40% involve the descending aorta. The prevalence at autopsy is roughly 3%, with a 2:1 male ratio. Most aneurysms are fusiform, although saccular aneurysms can arise, particularly in the setting of a penetrating aortic ulcer (PAU). The most common predisposing factors include systemic hypertension, atherosclerosis, and chronic obstructive pulmonary disease. Genetically mediated connective tissue disorders such as MFS, Loeys-Dietz syndrome (LDS), and Ehlers-Danlos syndrome (EDS) type IV can commonly lead to thoracic aneurysm formation [46].

Natural history

The natural histories of the dilated aortic root and mid-ascending aorta have traditionally been left entangled in a single analysis. Even the guideline documents perceive these two segments as a single unit although they differ significantly in anatomy, embryology, and in physiologic function [47]. Thoracic aortic aneurysm's can be due to one of several aetiologies. The natural history of TAA is one of progressive expansion, the rate of which depends upon the location of the aneurysm and its underlying cause.

Familial TAAs grow faster, up to 2.1 mm/year (combined ascending and descending TAA). Syndromic TAA growth rates also vary. In patients with MFS, the TAA growth is on average at 0.5 –1 mm/year, whereas TAAs in patients with LDS can grow even faster than 10 mm/year, resulting in death at a mean age of 26 years. [48, 49, 50]. There is a rapid increase in the risk of dissection or rupture when the aortic diameter is 6 cm for the ascending aorta and 7 cm for the descending aorta. Although dissection may occur in patients with a small aorta, the individual risk is very low [51].

Ascending aorta

Thoracic aortic aneurysms are asymptomatic dilatation of the thoracic aorta that confer a predisposition to dissection, which is often fatal. While the majority are associated with hypertension and atherosclerosis, a significant proportion are due to mutations in proteins within the aortic wall. Some of these mutations result in clinically identifiable syndromes such as MFS or EDS however some have no discernible physical features at all, other than aortic dilatation. Despite the variation in the proteins affected by these genetic mutations, there is a unifying pathological endpoint of cystic medial degeneration [52].

Aortic root

The aortic root is a highly sophisticated and complex structure as shown above. Its optimal structure ensures dynamic behaviour in flow characteristics, coronary perfusion and left ventricular function, and therefore abnormalities greatly impact valvular and coronary function. The aortic root and the entire length of the aorta naturally increase in diameter with age and increasing body surface area [53].

Aortic root dilatation with or without aortic regurgitation is the most observed pathological change of the aortic root. Pathological aortic root dilatation (aneurysm) is most diagnosed in the second to fourth decades of life [53].

Aneurysms of the aortic root arise relatively deep within the heart and because of frequently associated complications, such as aortic insufficiency, present a more complicated problem than the more distal aneurysms of the ascending aorta [54]. Aortic root aneurysms appear in less than 1% of open-heart surgery patients, but they can cause aortic regurgitation, dissection, and rupture with high morbidity and mortality. Progressive dilatation of the aortic root is caused by medial degeneration and destruction of the elastic and collagen fibres and can also be associated with high blood pressure, high stroke volume, and inflammatory diseases. Medial degeneration is a fated trend caused by the primary syndrome such as MFS, EDS, or LDS. In most patients with these syndromes, the primary dilatation develops at the aortic root, especially at the aortic sinus [53].

There are currently no documented studies that differentiate the biomechanical characteristics of the aortic root from that of the ascending aorta.

Aortic stenosis

Aortic stenosis (AS) is the narrowing of the heart's AV, which obstructs blood flow from the heart and to compensate, the heart needs to work harder to pump enough blood to the body [55]. Aortic stenosis has been estimated to occur in 0.3% to 0.5% of the general population and 2% to 7% of individuals older than 65 years of age. The prevalence of severe AS, for which intervention should be considered, may be as high as 3% to 4% in older adult (>75 years of age) populations [56].

The classic symptoms of AS are angina, syncope, and dyspnoea. Aortic stenosis is usually recognised by the presence of a harsh systolic ejection murmur that radiates to the neck. Other reported signs include:

- Delayed timing of carotid upstroke
- Reduced carotid upstroke volume, because the stenotic valve steals energy from the flow of blood as it passes the valve
- A forceful apical beat, which along with the carotid signs, supports the obstruction that exists between the LV and the systemic circulation

- A2 component of S2 is lost, because the severely stenotic AV barely opens, and there is little valve movement upon closing
- A soft single second heart sound
- S4 is usually present in patients in sinus rhythm, reflecting impaired filling of the thickened, noncompliant LV

Calcific AV disease (Figure 1.25) which includes aortic sclerosis and AS, has come to be recognised as an active process, based on: (1) epidemiologic studies demonstrating associations of specific risk factors with increased prevalence or rate of progression of AV disease; (2) identification, in valve lesions, of histopathologic features of chronic inflammation, lipoprotein deposition, renin-angiotensin system components, and molecular mediators of calcification; and (3) identification of cell-signalling pathways and genetic factors that may participate in valve disease pathogenesis.

Calcific AV disease is identified by thickening and calcification of the AV leaflets in the absence of rheumatic heart disease. It is divided, on a functional basis, into aortic sclerosis, in which the leaflets do not obstruct left ventricular outflow, and AS, in which obstruction to left ventricular outflow is present [57].

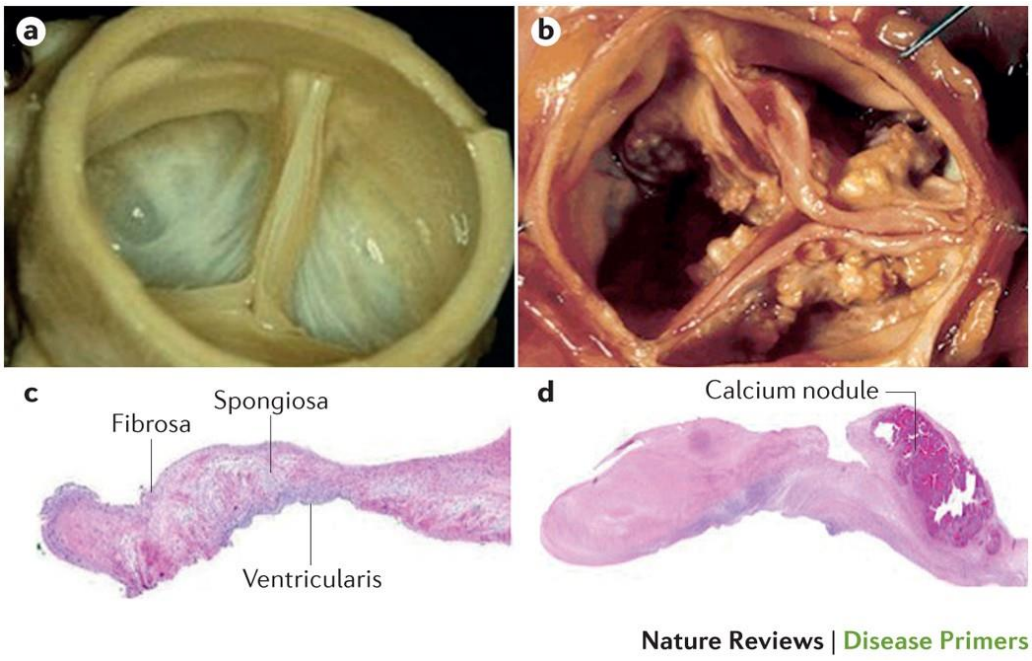

Figure 1.25: Photographs of a normal AV (part a) and an AV with severe calcific AS (B). Histopathological section of a normal AV with haematoxylin staining showing the trilaminar structure of the valve from top to bottom (C). Histopathological section of a valve with severe calcific AS with haematoxylin staining showing the presence of fibrotic material and a calcified

nodule. The tissue is thickened by the excess of fibrotic material, and the calcified nodule, located in the fibrosa, contributes to alter the normal architecture of the leaflet (D) [58].

Bicuspid Aortic Valve

Bicuspid aortic valve (BAV) disease is among the most common of congenital defects, affecting 1%–2% of the population [7]. Bicuspid valves are characterised by the presence of only 2 complete commissures (though an incomplete third commissure is often present) and unequally sized leaflets.

Aortic valve abnormalities are associated with aneurysms of the ascending aorta, ventricular septal defects, aortic coarctation, and dissection of the carotid and vertebral arteries, which are not all easily attributed to secondary haemodynamic effects of valvular irregularities. Craniofacial defects are also associated with BAV, suggesting an underlying relationship to neural crest, which contributes to craniofacial mesenchyme. Furthermore, numerous pathological studies have demonstrated noninflammatory degeneration of neural crest–derived smooth muscle cells in the ascending aorta and aortic arch of patients with bicuspid aortic valves, even those without aneurysm formation, which is often characterized as cystic medial necrosis [8].

There have been several large (and small) studies that have sought to establish the genetic causes of BAV and AS in the human population. There are many good examples of mouse gene knockouts that result in valve dysplasia and/or BAV and in other species such as the Syrian hamster [54]. These animal models have given essential insights into how disruption of different developmental mechanisms, genes, and signalling pathways can lead to different types of BAV and valve dysplasia [Figure 1.26].

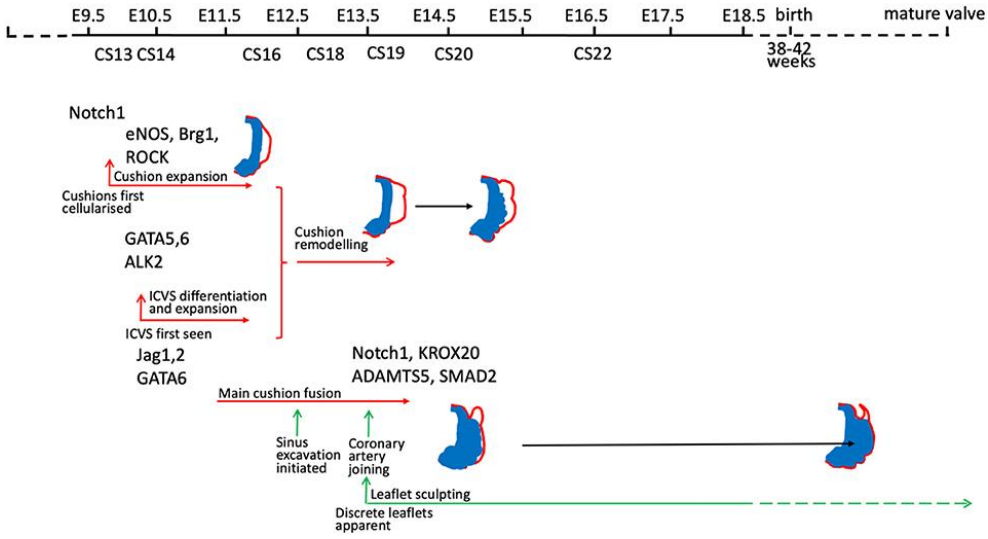

Figure 1.26: Timeline illustrating the timing of gene activity (based on developmental process disrupted) in mouse models of BAV. Mouse models included are those for which mechanistic

studies have been carried out to understand why BAV develops. Notch 1; eNOS; Brg1; ROCK; GATA5; GATA6; ALK2; Jag1/2; Krox20, ADAMTS5/Smad [54].

The genetic pathways that have been identified include: ACTA2, SMAD6, NOTCH1, ROB04, GAT5, TGFBR1/2, FBN1, ADAMTSL1, ADAMTS-4, NOS3, and chromosomes 18q, 5q, and 13q [79, 80].

The rarer variants of NOTCH1, GATA5 and FBN1 were correlated with 4–10%, 2.6% and one in eight BAV patients, respectively. More common variants in FBN1 were also found to be associated with BAV/TAA [55].

BSA and aortic size influence

Indexing aortic size in relation to patients' height and body surface area is one of the parameters that has emerged that could aid in timing prophylactic surgical repair more accurately in TAA patients. [56].

It has been reported that relative aortic size (aortic size indexed to the body surface area of a patient) was a more accurate predictor of the risk of aortic rupture, dissection, or death than aortic size alone [57].

As a predictor of adverse aneurysmal outcomes, aortic diameter indexed to body stature remains relevant and superior to any other criterion, except in rare cases of symptomatic aneurysm presentation (with pain) when surgery is required, and size becomes irrelevant [56].

1.4.2 Histopathology

1.4.2.1 Ascending aorta aneurysm

Thoracic aortic aneurysms (TAA's) pathology was originally described from autopsy cases. One of the earliest papers to describe the histopathology of resected aortic aneurysms from intraoperative specimens was in 1977 [63]. From 1969-77 Pomerance and colleagues resected 63 aneurysm samples from a group of men and women of varying ages and aetiology. Their most common histological finding was cystic medionecrosis (CMD) with and without elastopathy in 71.4%, aortitis in 22%, varying defects in elasticity, and transmural defects that seemed to predispose to partial dissection and rupture. They reported marked loss of elasticity without linear arrangements and elastic tissue loss disproportional to the amount of acid mucopolysaccharide. Thick layers of connective tissue occupied the intimal layers that was fibroelastic with high mucopolysaccharide. Adventitial fibrous thickening was also reported. Partial dissections were reported in five cases. Atheroma formation as the primary pathology was also reported in 2 cases [63].

Kilma and colleagues [64] evaluated the morphological abnormalities in TAAs in 339 patients. The primary comparisons were made between MFS and non-MFS patients. They evaluated the degree of elastic fibre fragmentation, cystic medial change, medial fibrosis, and vasa vasorum thickening. They found that elastic fragmentation and cystic medial change was inversely correlated with increasing ages of patients; as was medial necrosis, fibrosis, and atherosclerosis. Dissection was more commonly seen with medial abnormalities than with atherosclerosis. There was no direct correlation between aneurysm size and pathology. Savunen and colleagues [65] assessed the histological characteristics of 44 patients with annulo-aortic ectasia. They found greater cystic change, elastic fragmentation, fibrosis, and disappearance of smooth muscle cells in the aortic media in ectasia patients than in control specimens of normal aorta taken at biopsy. This study mirrored the results of Kilma and colleagues with no direct link between aneurysm size and pathology. The 2010 guidelines into the management of TAA's [66] characterised medial degeneration as disruption and loss of elastic fibres and increased deposition of proteoglycans. They describe loss of smooth muscle cells in the media, and the presence of inflammatory cell infiltrate. The aortic media is described as showing focal hyperplasia, with random smooth muscle cell orientation.

In a study by Butcovan et al (2019), medial degeneration was the leading histopathological diagnosis in TAA. The severity of lesions was graded as follows: Mild (8% of cases), moderate (44% of cases) and severe (31% of cases) (Figure 1.27) [67].

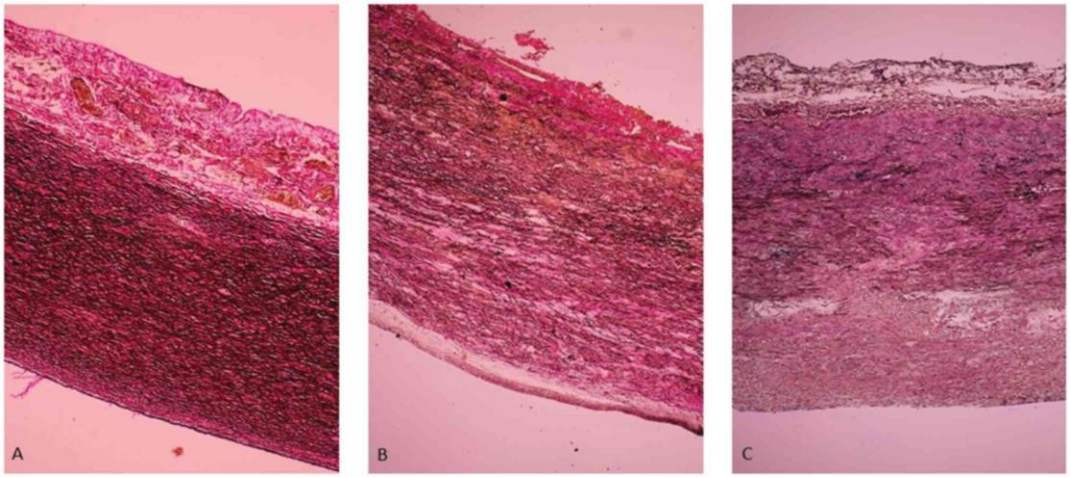

Figure 1.27: Aortic medial degeneration. Tissues of (A) grade 1, (B) grade 2 and (C) grade 3, were determined via Elastic Van Gieson's staining (magnification 100x) [67].

The pathology involving TAA's has been described as involving all layers of the aortic wall [65]. Amalinei and colleagues in 2013 reported degradation of elastin [Figure 1.28], and collagen fibres, cystic medial change [Figure 1.29], and fibrosis, reduced vascular smooth muscle cells, lymphocyte infiltration, and vasa vasorum thickening [36]. They also reported haemorrhage associated medial

layer splitting [Figure 1.29] due to elastic fragmentation and fibrosis in the dissected specimens. They reported on previous studies identifying the relationship between size and rupture risk [68, 69].

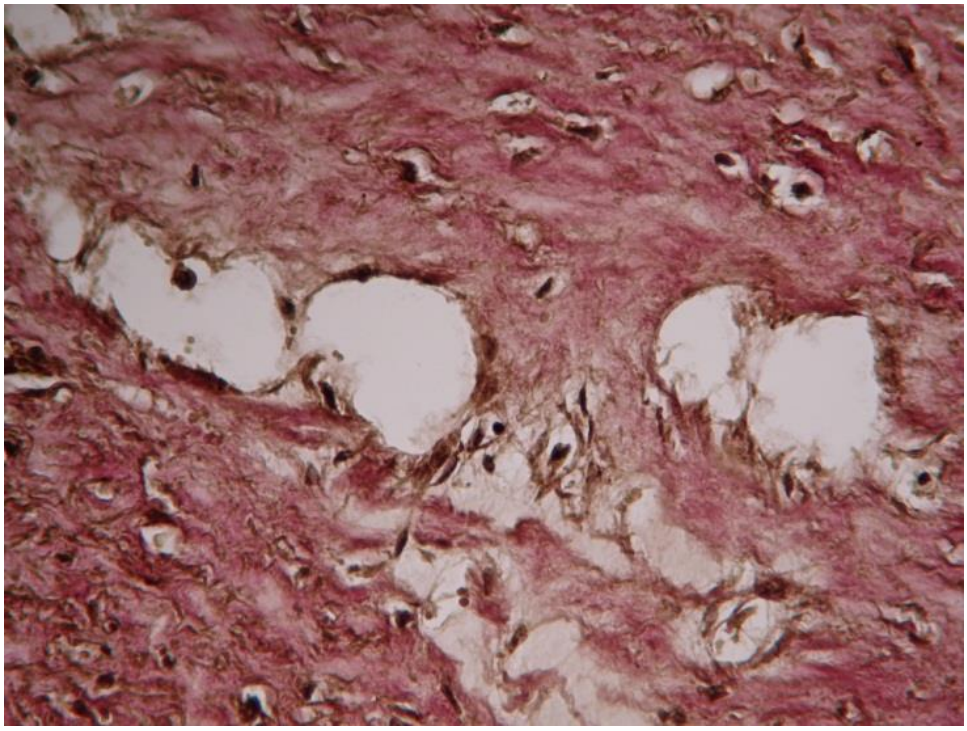

Figure 1.28: Elastic fibre fragmentation with Elastic-van Gieson (EVG) staining at 40x magnification. Image adapted from [36]**.**

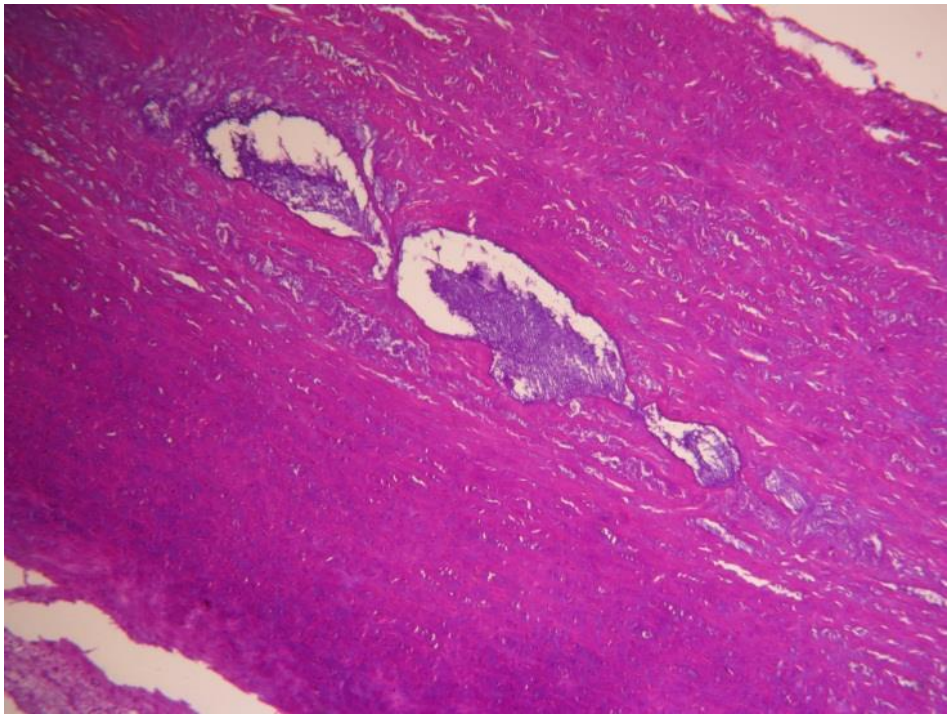

Figure 1.29: Cystic medial degeneration with hemoxylin and eosin (H&E) staining at 40x magnification. Image adapted from [36].

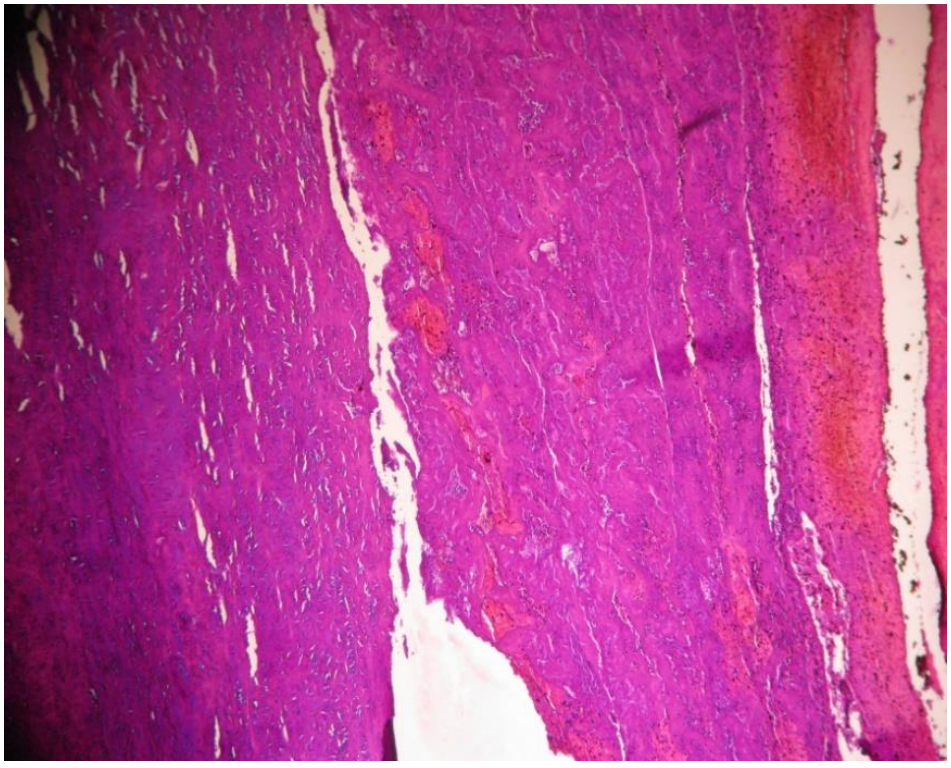

Figure 1.30: Dissecting aneurysm with H&E staining at 40x magnification. Image adapted from [36].

Determining the extent of these pathological changes depends on grading the specimen, and this has been done in a variety of ways in the field of pathology. Cystic changes between the media layer characterised by H&E positive basophilic material or alcian blue positive staining are classified into three grades. Grade 1 shows minute cysts with up to five foci of elastic fibre degeneration extending two to four lamellae within the width of the media: Grade 2 involving the maximum width of one lamella unit extending to more than five foci, and Grade 3 extending more than the width of a lamellar unit and involving the smooth muscle tissue [36, 65].

Elastic fibre degeneration types were described by Doerr and colleagues in 1974 [70]. They described two types of degeneration; Microcystic (Gsell-type), and Disseminated cystic (Erdheim-type). It was described that the elastic fibre degeneration was directly related to an increase in collagen content [Figure 1.31] [36, 37, 40, 41, 65].

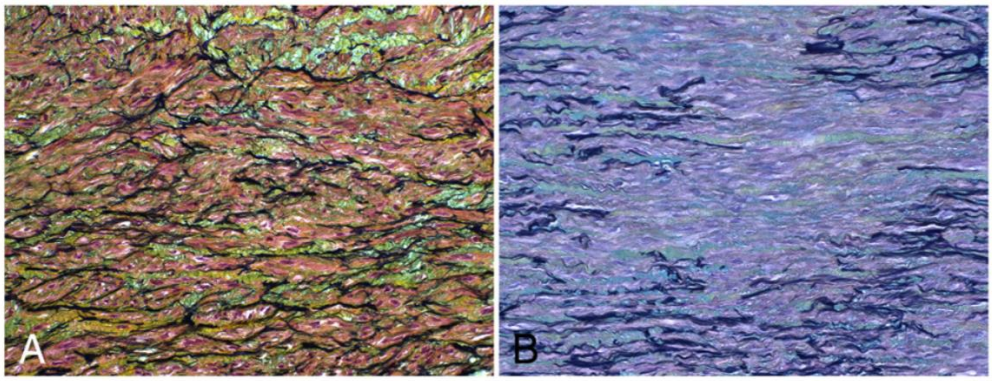

Figure 1.31: Elastic fibre fragmentation and/or loss. (A) Fragmentation of the elastic fibres, where they no longer extend across the length of the image, is seen. (B) Complete loss of elastic fibres can occur (Movat's pentachrome, 400x) [40].

Fibrosis was also graded (21). Grade 1 involves less than 1/3 of the medial thickness, Grade 2 extends more than 1/3 and no more than 2/3 of the media thickness; and Grade 3 involves more than 2/3 of the aortic media thickness. Humphrey and colleagues (2008) in the examination of abdominal aortic aneurysms described a process whereby the smooth muscle is progressively lost [Figure 1.32] [38, 40].

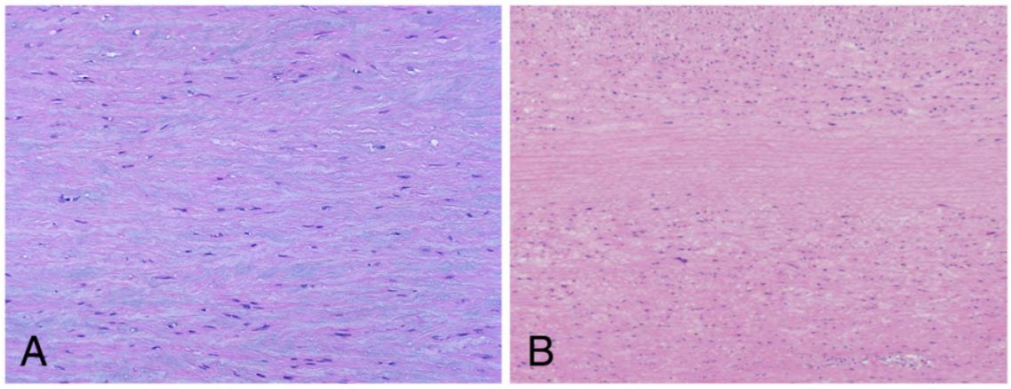

Figure 1.32: Smooth muscle cell nuclei loss. Smooth muscle cells, as noted by their nuclei on an H&E stain, can be lost in a (A) patchy or (B) band-like fashion (H&E, 200x, 160x) [40].

1.4.2.2 Aortic root aneurysm

The pathology of the aortic root aneurysms was first described in 1966 by Najafi and colleagues as a form of CMD, syphilis, and arteriosclerosis; with medionecrosis being the most common cause. [58]. In a case report described of a 49-year-old male with aortic root enlargement, the resected pathology revealed CMD. It has been described as a degenerative and non-inflammatory process [28]. The degenerative changes include medial fragmentation, smooth muscle cell necrosis, and elastic fibre fragmentation with cystic spaces in the media filling with mucoid material. The elastic fibre network breaks down and the connections with the complex collagen networks are lost [Figure 1.33 and 1.34].

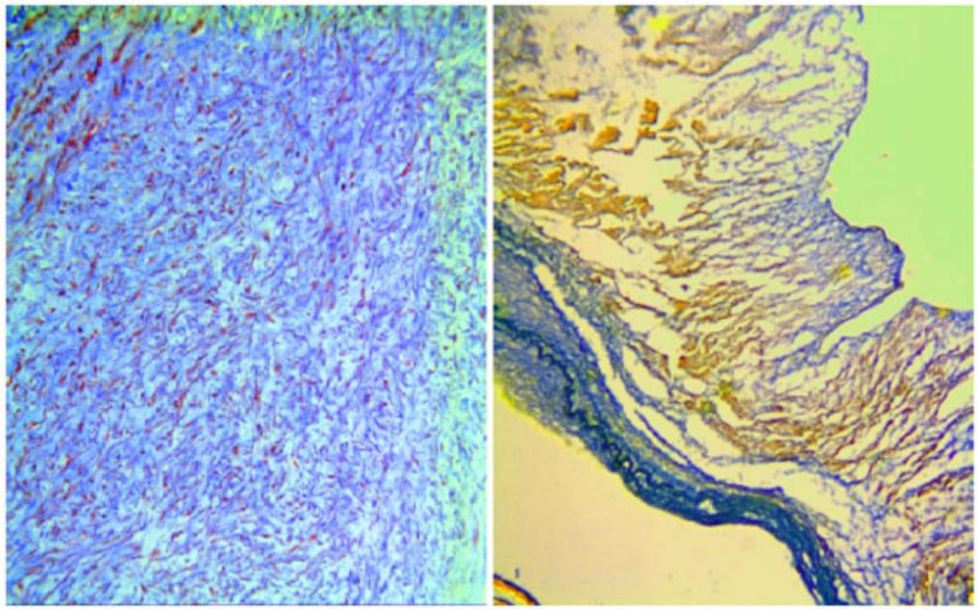

Figure 1.33: Marfan's aortic tissues showing cystic medial necrosis with (Left image) smooth muscle cell fragmentation and more collagen deposition, Masson 200x; and (Right image)

proliferation and disruption of the intima (blue), and smooth muscle cell fragmentation (yellow) and collagen deposition (red) in the media. VG-Victoria blue bichrome staining×100 [71].

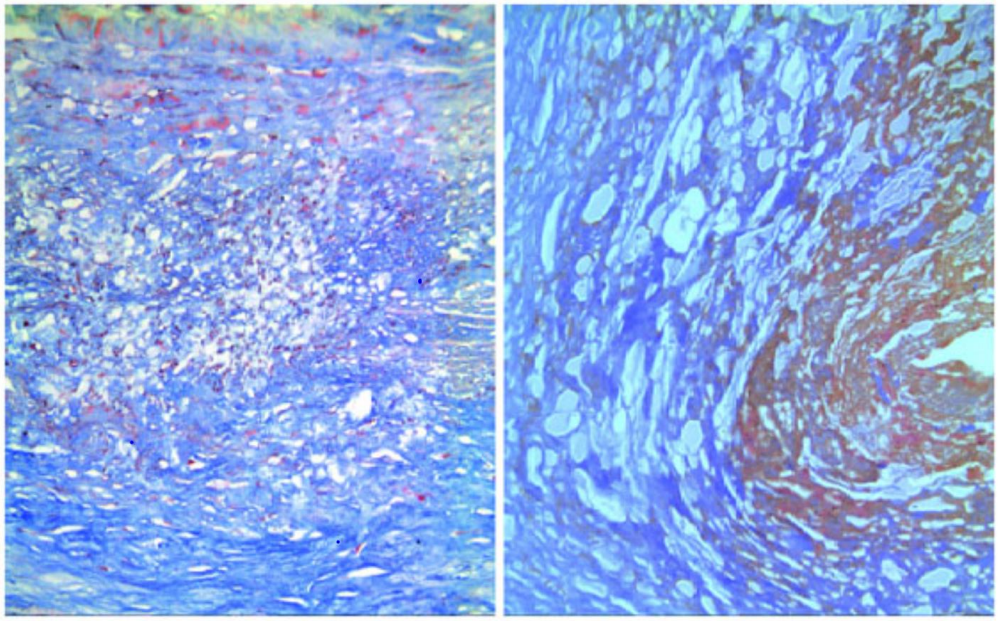

Figure 1.34: Cystic medial necrosis of ascending aortic aneurysm of (left image), showing much collagen deposition in the intima and smooth muscle cell fragmentation with few collagen and cystic-like lesions in the media, and (right image) ascending aortic aneurysm with aortic insufficiency and stenosis, showing degenerative disruptions elastic fibres and smooth muscle cells with few collagen but more cystic-like lesions in the media. Masson 200x [71].

The collagen types change significantly; with collagen I and III decreasing and collagens XI and V increasing. Smooth muscle cells are lost, and basophilic material increases in a similar way to ascending aortic aneurysms [28, 29].

Histopathological tissue analysis has historically represented the definitive method of disease presence and disease grading [72].

1.4.2.3 Collagen content in the aneurysmal aorta

Collagen appears to play an important role in aortic aneurysms. There are studies reporting that the total amount of collagen is increased in the aneurysmal aorta. Whittle and colleagues [73] examined dissecting aneurysms, with aortic sites involving dissections compared with controls. They concluded that in the case of the dissecting aneurysms, there was a significant increase in the amount of collagen and a significant decrease in the collagen concentration. This made the aortic wall weaker and less able to withstand the mechanical stresses constantly imposed upon it.

Carmo and colleagues [76] confirmed decreased elastin content in aneurysmal walls with a corresponding increase in collagen cross-links. They concluded that since the total collagen markers were decreased, it is reasonable to suggest that in aneurysmal aortic walls, old collagen accumulates cross-links while new collagen biosynthesis is somehow defective.

In contrast, Borges, and colleagues [77] found that collagen is reduced and disrupted in human aneurysms and dissections of the ascending aorta. They showed a decrease in collagen content that could be related to a weakness of the wall underlying the diseases.

It has also been observed that the collagen proportion (% cross-sectional area) in the media layer specifically of the ascending thoracic aorta (ATA) varied with disease conditions [78]. In ATA dissection, the collagen proportion was decreased from 33 ± 12% in the inner half of the media to 19 ± 12% in the outer half ($p < 0.01$). In the wall of ATA aneurysm, collagen proportion did not differ significantly ($p = 0.71$) between inner (20 ± 10%) and outer halves (18 ± 12%). In control ATA, the collagen proportion was decreased from 50 ± 13% in the inner half of the media to 40 ± 8% in the outer half ($p = 0.04$). The collagen proportion in the wall of ATA dissection and in the wall of ATA aneurysm was less than control ($p < 0.01$). The homogeneous proportion of collagen seen in the media layer of ATA aneurysm could be associated with an overall weakening of the wall that would lead to aneurysmal dilation. In the media layer of ATA aneurysm and dissection, dramatic morphological changes in collagen bundles were observed with collagen fibres being thin and having more scattered fibres. In contrast, in the control ATA, thick collagen fibres and bundles were observed with a parallel arrangement in the media as well as a few thin collagen fibres dispersed perpendicularly [78].

1.4.2.4 Elastin content in the aneurysmal aorta

In aortas from patients with MFS, elastin was deficient in cross-linking and the content of elastin was decreased by almost 50%. This was further validated in a report also showing elastin content was decreased by 50%, but specifically in the media of MFS aorta with structural alterations of elastin fibres being characterised by enlarged interlaminar spaces (between elastin laminae) and loss of interlaminar elastin fibrils. This loss of elastin content and decrease in cross-linking could explain the higher prevalence of MFS patients to aneurysm, because the degradation of elastin could cause a release of significant compressive pre-stresses within the wall and subsequently lead to diameter enlargement [78].

Common structural changes in aneurysm tissue include an early loss of elastin and smooth muscle cells. A 90% reduction in elastin and indicators of excess, aged collagen, and impaired new collagen synthesis are reported in aneurysmal specimens compared to non-aneurysmal abdominal aortic tissue. It has also been shown that there are significant changes in the media layer including a

fragmentation of the elastic laminae and fibres. Regarding the mechanical properties, human aortic aneurysm tissue shows increased elastic modulus and anisotropy compared to healthy tissue [79].

1.5 Pathophysiology

1.5.1 Pathophysiology of thoracic aorta aneurysms and aortic root aneurysms

Aneurysms of the ascending thoracic aorta most often result from CMD, which leads to weakening of the aortic wall, resulting in aortic dilatation and aneurysm formation. Cystic medial degeneration occurs normally to some extent with aging, but the process is accelerated by hypertension [80]. Unfortunately, much of the scientific discussion regarding ascending aorta and aortic root aneurysm pathophysiology and pathogenesis is blurred into one ascending aorta region making distinction difficult.

1.5.1.1 Thoracic aorta

Pathophysiology of the thoracic aorta is poorly understood, with often clinically silent and fatal rupture if undetected. Improved definition of the structure and function of the normal aortic wall, coupled with the discovery of genetic mutations in key regulatory molecules, have contributed to a more detailed understanding of the pathophysiology of syndromic, familial, and sporadic TAAs [81].

Genetic connective tissue mutations

TAAs are classified as syndromic, familial, or sporadic:

Syndromic TAA

Syndromic TAA's are aneurysms that are associated with multi-faceted syndromes such as MFS, LDS, and EDS (Type IV), as well as autosomal-dominant familial patterns of inheritance. Investigation into the consequences of these known mutations has provided insight into the cell signalling cascades leading to degenerative remodelling of the aortic medial extracellular matrix (ECM) with TGF-β playing a major role [81, 82]. In other cases, such as BAV and Turner syndrome (TS), TAAs are a possible manifestation.

Analysis of cytogenetic screening studies indicate that TS occurs in approximately 1/200 conceptions but only 1/2000 live female births with congenital cardiovascular defects leading to a high rate of foetal demise [74]. Common congenital defects in surviving girls and adults with TS include BAV (~30%) and aortic coarctation (~12%). Depending on the definition, the prevalence of aortic dilatation ranges from 4% to 42%, and aortic dissection is reported to occur six times more often compared with the general population [74].

There have been reports of a high rate of aortic dissections in TS, including patients without predisposing factors such as BAV, and there seems to be a generalised dilatation of major vessels in women with TS, including the aorta, brachial, and carotid arteries [75].

Familial non-syndromic TAA

These TAAs follow a familial pattern of inheritance, often autosomal dominant, with decreased penetrance (especially in female family members) and variable expression [81]). Six different genetic loci have been recognised in families with familial non-syndromic TAAs, but only three genes have been identified: TGFBR2 in TAA2, ACTA2 in TAA4 and MYH11 in familial TAA and patent ductus arteriosus. The remaining loci are 5q13-14 (TAA1, about 10–30% of familial nonsyndromic TAAs), 11q23.3-24 (FAA1, less than 5% of familial nonsyndromic TAAs), and 15q24-26 (TAA3, about 10–20% of familial nonsyndromic TAAs) [81].

Sporadic TAA

These aneurysms occur in isolation and do not show any familial transmission, and remain somewhat misunderstood. Sporadic TAAs can originate from 'degenerative', inflammatory (giant cell arteritis), autoimmune (Takayasu arteritis, rheumatoid arthritis, or Reiter syndrome), infectious (syphilis or tuberculosis) or traumatic conditions [81]. The effects of these conditions on the aortic root are discussed in more detail below.

Degenerative changes in the elastic media

Thoracic aortic aneurysms are most associated with degeneration of the elastic media due to CMD with elastic fibre fragmentation and smooth muscle loss. This is a normal process of ageing; acceleration of this process can occur resulting in increased risk of aneurysm progression and rupture [83].

Atherosclerosis and Acute dissection

Although atherosclerosis less commonly affects the ascending aorta, aortic media atherosclerosis can cause disruption of elastic fibres and smooth muscle cells resulting in atheroma's and subsequent weakening and destruction of the aortic wall. If the aorta does not dissect at this time, then aneurysmal dilatation will result. Evolution of these aneurysms are commonly from an area of dissected false lumen [83].

Other

Valve malformations such as BAV, infections (mycotic aneurysms), and arteritis are seen specifically within the aortic root apparatus and although they can affect the ascending thoracic aorta, are discussed in detail below.

Pseudoaneurysms often develop following chest trauma, cannulation injuries, or along suture lines of the aorta postoperatively [83].

Key structural components

In addition to their structural role, the cells and proteins in the aortic wall have important regulatory functions that maintain homeostasis [81].

Vascular smooth muscle cell (VSMC)

Mutations involving cytoskeletal proteins in the VSMCs are involved in TAA formation, probably through loss of direct feedback mechanisms that lead to loss of shape and alignment, and abnormal signalling and synthesis of ECM proteins. This results in VSMC apoptosis and disarray, and elastin fragmentation which are somewhat characteristic of TAA aneurysms [81].

Collagen

As collagen is a vital and prominent structural component of the thoracic aorta, mutations in genes encoding collagen fibres have direct structural and functional consequences that could contribute to aneurysm formation. Mutations in the COL3A1 gene encoding type III collagen are associated with vascular type EDS [81].

Elastin

Similarly, elastin exists as a vital structural component of the ascending aorta, with elastin abnormalities resulting in an uncontrolled fibro-cellular proliferative state of VSMCs with associated downstream effects. Mice that were deficient in elastin die soon after birth because of severe occlusive disease of the aorta, characterised by unregulated VSMC proliferation and fibrous deposition. Further studies with loss-of-function mutations of one elastin allele result in supravalvular stenosis and Williams syndrome. These effects are characterised by discrete stenosis in the aorta and other arterial beds caused by subendothelial proliferation of VSMCs despite normal endothelial function and the absence of inflammatory or oxidative stimuli [81].

Fibrillin

Fibrillin 1 mutations are associated with MFS, and is a key structural component that contributes to the strength of the aortic wall by forming a lattice around elastic fibres. Fibrillin 1 has a crucial role in the activity of growth factors and other microfibrillar proteins in the ECM, such as TGF-β1 and bone morphogenic proteins, and activates cell signalling pathways by binding to integrin receptors on fibrillin. The precise effect of VSMC–fibrillin interactions remains unclear, but they are thought to provide positional signalling to the cells.

Fibulin

Fibulin 4 mutations in humans present predominantly as aortic and arterial aneurysms and tortuosity, as well as some skeletal features such as joint hypermobility, and both fibulin 4 and 5 have been shown to be integral in aortic wall structural integrity. Studies have shown that mice that lack fibulin 5 expression demonstrate marked elastinopathy, characterised by aortic tortuosity, loose skin, and emphysematous lungs, but no aortic dilatations have been described in humans [81].

Major regulatory pathways

Ontological regulation

The transforming growth factor β1 pathway is important in matrix regulation in health and disease, and increased activity is a key component of various forms of TAA's. Transforming growth factor β1 stimulation of neural-crest-derived VSMCs has resulted in a significant increase in DNA synthesis, cell proliferation, activation of the protein kinase C signalling pathway and collagen production implicating aortic root and thoracic aorta development [81].

Mechanical regulation

Mechanical cues such as stress and strain on the thoracic aorta during each cardiac cycle have a direct effect on structure and function of cells in the aortic wall, characterised by changes in cell alignment, migration, proliferation, and synthesis.

Aneurysmal aortic specimens show reduced collagen in areas of dilatation, which could be attributed to higher levels of matrix metalloproteinase (MMP) induced proteolysis, and VSMC induced apoptosis, that was significantly increased at the aortic convexity (the site of highest stresses), compared with other areas of the ascending aorta [84]. This demonstrates the importance of the local mechanical environment and the role of activated mechanotransduction pathways in mediating cellular and matrix responses [81].

Dysfunction of one or more components of the cytoskeleton–receptor–extracellular matrix complex can lead to structural and functional dysregulation of aortic wall properties [81].

Wall stress

As the thoracic aorta dilates, the pattern and magnitude of shear can vary significantly along its surface, translating into altered paracrine signalling from endothelial cells to underlying VSMCs, leading to a change in proliferative, contractile, and synthetic properties.

There is a growing body of literature suggesting that stress measurement in the aortic wall may aid in the identification of aneurysms that are at high risk of rupture. Ruptured abdominal aortic aneurysms (AAA) have been shown to have higher peak wall stresses than unruptured aneurysms [85]. A patient-specific study demonstrated that peak wall stress was 13% more sensitive and 12%

more specific in predicting ruptured AAAs than maximum diameter alone [86]. Li [87] correlated aneurysm shoulder stress with growth rate, showing that individual aneurysms with higher shoulder stresses were associated with increased AAA expansion. Despite the relative success of biomechanical modelling techniques in stratifying AAA rupture risk, these techniques have not commonly been applied to TAAs [88].

Biomechanical signalling

TGF-Beta1

TGF-β1, a member of a large family of cytokines, has a central role in cardiac and vascular morphogenesis and in maintaining ECM homeostasis. It has a role in collagen and elastin production, and a critical opposing role leading to matrix degradation through increased production of plasminogen activators and release of MMPs 2 and 9 in the ECM. Mutations in TGF-β receptors have been linked to several conditions leading to TAAs, including LDS, and a familial non-syndromic form of TAA [81].

MMPs

Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) constitute a series of zinc-containing enzymes capable of matrix degradation, remodelling and processing of ECM proteins and adhesion molecules. Matrix proteolysis is one of the hallmarks of TAAs, and increased MMP expression is consistently observed in TAA specimens [81].

Inflammation and oxidative stress

Histological analysis of TAAs often shows the presence of inflammatory cells in the adventitia and media of the aortic wall. The outside-in theory proposed macrophage-dependent inflammation in the adventitia, and a direct role in the pathogenesis of aortic aneurysms (albeit abdominal aneurysms) [89].

Reactive oxygen species can increase the expression and activity of MMPs, as well as induce VSMC apoptosis, resulting in aortic wall weakening and elastolysis, but this research has focused on abdominal aortic aneurysms to date [81].

1.5.1.2 Aortic root

Aortic root dilatation pathogenesis, like TAA, is based on associated aetiologies.

When ascending aortic aneurysms involve the aortic root, the anatomy is often referred to as annuloaortic ectasia. Annuloaortic ectasia is a combination of:

1. Ascending aorta aneurysms
2. Dilatation of the SOV
3. Dilatation of the aortic annulus

Therefore, it would be considered as aneurysmal dilatation of both the aortic root and ascending aorta. It can occur as an isolated condition, or in association with a connective tissue disorder discussed below.

Genetic mutations

MFS

Animal studies demonstrated over-expression of TGF-β in the mitral valve preceding prolapse, the aorta associated with dilatation, skeletal muscle associated with myopathy, and the dura leading to ectasia. Mutations in TGF-β receptor 2 (TGFBR2) and TGFBR1 genes were identified in some patients with MFS phenotypes and subsequently implicated in the disease process in fibrillin 1 (FBN1) mutation negative individuals [90].

LDS

LDS subtypes are labelled 1–6 and associated with mutations in TGFBR1, TGFBR2, SMAD3, TGFB2, TGFB3, SMAD2 respectively, with aortic root dilatation being the hallmark of the clinical findings and is seen in approximately 80% of patients [90].

EDS

Vascular complications can be seen with different types of EDS; however, it is most seen in type IV (vascular or arterial ecchymotic type; vESD), characterised by an autosomal dominant mutation in COL3A1 (collagen, type III, α-1 gene) encoding type III procollagen. Up to 80% of these patients will suffer from a vascular complication before the age of 40 years [90].

Non-genetic causes

Idiopathic

Aortic root dilatation is associated with age, body surface area (BSA), height and gender. Age induced changes is based on the idea of cyclic stress, and how the aorta degrades through gradual mechanical decline of elastin proteins, and shear stress, which over a normal lifetime result in the degradation of elastic lamellae, resulting in arterial dilation and stiffening [90]. Further postulation is that age-associated reprogramming that is proinflammatory promotes progression of arterial disease. It is reported than men have a higher incidence of aortic root dilatation compared to women [90].

Hypertension

The relationship between hypertension and aortic root dilatation is not as well established as hypertension and aortic dissection.

Links have been established between genetic syndromes (Turner Syndrome) and increased risk of root dilatation with hypertension, and this has been attributed to a cyclic stress hypothesis which stated chronic shear stress and associated dilatation is correlated with increased pulse pressures leading to greater stress and dilatation [92]. However, inverse relationships have been reported [93], and therefore the mechanism of hypertension induced aortic root dilatation remains to be clearly established [90].

Infections

The incidence of aortic root mycotic aneurysms has greatly declined since the regular use of antibiotics, but some species are still reported including salmonella, staphylococcus, and streptococcus pneumonia [90, 94, 95, 96]. Other reported species have included mycobacterium tuberculosis, treponema pallidum, listeria, bacteroides, clostridium, and campylobacter [90].

Mycotic aneurysm development is generally saccular, and staphylococcal infections have been the predominant species to infect the AV and progress into aneurysms of the root [90].

Inflammatory disorders

Various inflammatory disorders associated with aortitis have reported aortic root dilatation. Ankylosing spondylitis was one of the first, and is thought to cause fibrous growth along the intima of the root leading to weakening of the tissues [97]. This combination is associated with significant aortic root abnormalities. Polychondritis is a multisystem inflammatory disorder with common cardiac involvement, specifically in the abdominal and thoracic aorta. Involvement of the root is associated with aortic regurgitation and high mortality. Takayasu arteritis is a chronic granulomatous large vessel vasculitis, that commonly affects the carotid and subclavian vessels, but can infiltrate the root resulting in aortic regurgitation and progressive aneurysm formation [98, 99]. Giant cell arteritis is also a chronic granulomatous large vessel vasculitis affecting the carotid, temporal, and vertebral vessels, but is also associated with thoracic aorta involvement, aortic regurgitation, aortic dissection, and aortic root involvement [100].

Left ventricular hypertrophy (LVH)

Although the mechanism is yet to be clearly determined, systematic reviews and imaging studies have shown a positive correlation between LVH and aortic root dilatation and increased risk of subsequent cardiovascular events [93].

1.5.1.3 Aortic stenosis

Aortic stenosis may be defined as narrowing of the AV, due primarily to a combination of progressive fibrosis and calcification of the matrix, with consequent increase in valve stiffness, progressive reductions in valve area and concomitant increases in left ventricular afterload and work [101].

Aortic stenosis is currently the most common form of valvular heart disease in the Western world in large part because the most frequently occurring form of AS develops predominantly in individuals of advancing age [102].

Narrowing of the AV in AS is due primarily to a combination of progressive fibrosis and calcification of the matrix, with consequent increase in valve stiffness, progressive reductions in valve area and concomitant increases in left ventricular afterload and work [102]. The causes of AS are shown in the table below (Table 1.1).

Aetiology	**Approximate frequency %**	**Associated features**
Ageing/calcific	**50-70**	**Increased risk of coronary events**
Bicuspid aortic valve	6-40	Dilatation of dissection of the aorta, involving the aortic root, ascending aorta, or aortic arch
Rheumatic	2-11	Mitral valve almost always affected as well
Unicuspid aortic valve	<1	Dilatation or dissection of the aorta, involving the aortic root, ascending aorta, or aortic arch
Post-endocarditis	<1	Extra-cardiac embolic phenomena

Table 1.1: Causes of AS. Wide frequency range generally reflects the age group(s) assessed by individual studies as well as population subgroups studied [101].

AS exerts a pressure overload on the left ventricle (LV). Normally, pressure in the LV and aorta are similar during systole, as the normal AV permits free flow of blood from LV to the aorta. However, in AS the stenotic valve forces the LV to generate higher pressure to drive blood through the stenosis, causing a pressure difference (gradient) from the LV to the aorta. The LV compensates for this pressure overload by increasing its mass (LVH) [101].

The main pathogenetic determinants in AS were always considered to be atherosclerosis associated with old age, male sex, hypertension, smoking etc, however >50% do not have significant atherosclerosis so therefore other pathogenetic mechanisms have been discussed including:

- Fibroblast activity and fibrosis
- Reactive oxygen species
- Pro-inflammatory and pro-fibrotic processes causing calcification
- Nitric oxide system
- Renin-angiotensin aldosterone system (RAAS)

Aortic stenosis is a complex active process, involving valvular endothelium, fibroblasts, and ECM. The process is characterised by inflammatory activation and lipid deposition within valve lesions. There is extensive valvular matrix remodelling and fibrosis with increased production of MMP-1 and 2, TGF-β1, interleukin-1 beta (IL1-β) and tumour necrosis factor alpha (TNFα). There is extensive evidence for increased production of Angiotensin II, a major pro-inflammatory and pro-fibrotic mediator, within stenotic valves. This would lead to further fibrosis and calcification. Impaired activation of anti-calcific modulators, such as fetuin-A and Matrix Gla protein (MGP), is also important in AS. There is concurrent increased in oxidative stress and evidence of impairment of the nitric oxide system as well as associated systemic endothelial dysfunction [101].

A further description describes AS an active process in which hypercholesterolaemia initiates endothelial dysfunction in aortic valves, with upregulation of oxidative stress and inflammatory processes leading to plaque formation, as well as a switch to an osteogenic phenotype with valve mineralisation [103].

A postulated schematic [Figure 1.35] describes the mechanisms contributing to the AV lesion formation. Initially, there is inflammatory infiltration of T-lymphocytes and macrophages, along with lipid accumulation. Subsequent interactions between chemical stimuli result, and disruption of valvular homeostasis through pro- and anti-fibrotic mechanisms. In the later stages of AS, cytokine release and angiotensin II promote ECM protein secretion at early stages of mineralisation which in turns begin the build-up of bone-like calcific nodules on the AV, further restricting leaflet mobility [101].

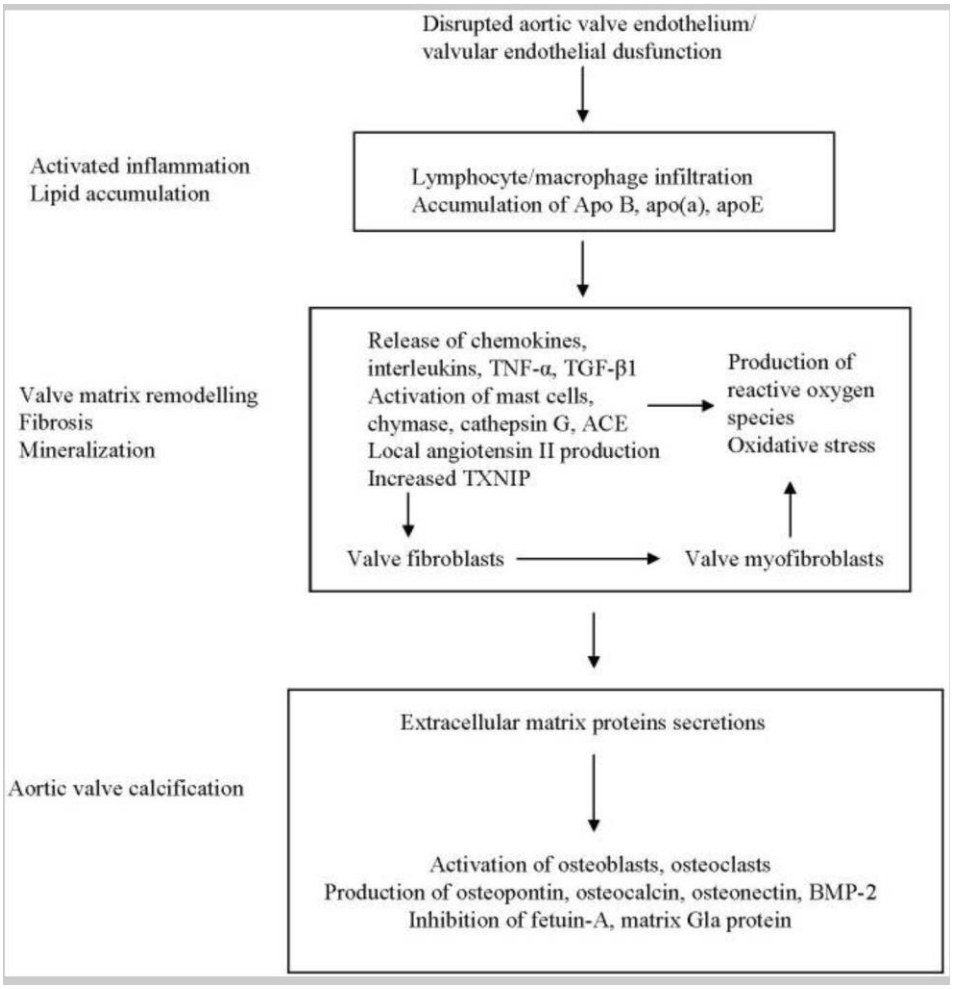

Figure 1.35: Diagram of postulated mechanisms underlying AV lesion formation [101].

1.6 Clinical Disorders

1.6.1 Clinical analysis of the pathological aorta and aortic root

1.6.1.1 Colour deconvolution

Colour deconvolution (CD) is a tool used in histological analysis that separates stains into their component parts. To overcome the difficulty in isolating certain proteins in histological analysis, Ruifrok and colleagues developed a technique of CD [104]. Traditional CD uses matrix inversion to change the Red, Green, and Blue (RGB) channels of an image into a new domain that is representative of reference colours [Figure 1.36]. Colour deconvolution quantification is provided in the imaging software (Image J, National Institutes of Health, LOCI, University of Wisconsin, US).

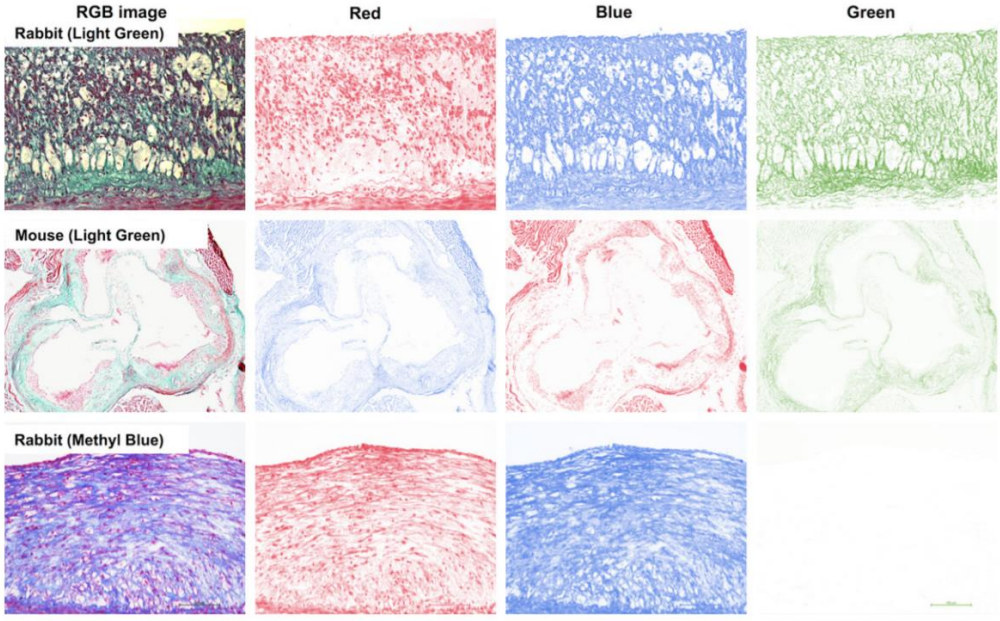

Figure 1.36: Images of atherosclerotic lesions processed by CD. The original RGB image was split into its red, blue, and green components. Image adapted from [105].

Collagen fibres in traditional histological staining have been identified by Masson's trichrome (MT) staining for more than 80-years [105]. Masson's trichrome staining selectively stains collagen, collagen fibres, fibrin, muscles, and erythrocytes. It used three stains hence the term trichrome (Weigert's Hematoxylin, Biebrich scarlet-acid fuchsin solution, and Aniline blue). Weigert's Hematoxylin, an iron hematoxylin dye is used to stain the nuclei. This dye is resistant to decolourisation by acidic staining solutions. Biebrich scarlet-acid fuchsin solution stains all the acidic tissues such as the cytoplasm, muscle, and collagen. Phosphomolybdic or phosphotungstic acid is used as a decolourising agent, making the Biebrich Scarlet-acid fuchsin diffuse out of the collagen fibres, leaving the muscle cells staining red. Aniline blue stains the collagen, and 1% acetic acid is added to show a difference in the tissue sections. The collagen fibres stain blue and the nuclei stain black, with a red background [Figure 1.37]. The three main colours often localise in the same area and therefore it is difficult to analyse or quantify tissues stained in MT stain. Colour deconvolution allows for collagen fibre quantification within a sample stained with MT and has been shown to be effective in studying atherosclerosis in human samples to date [105].

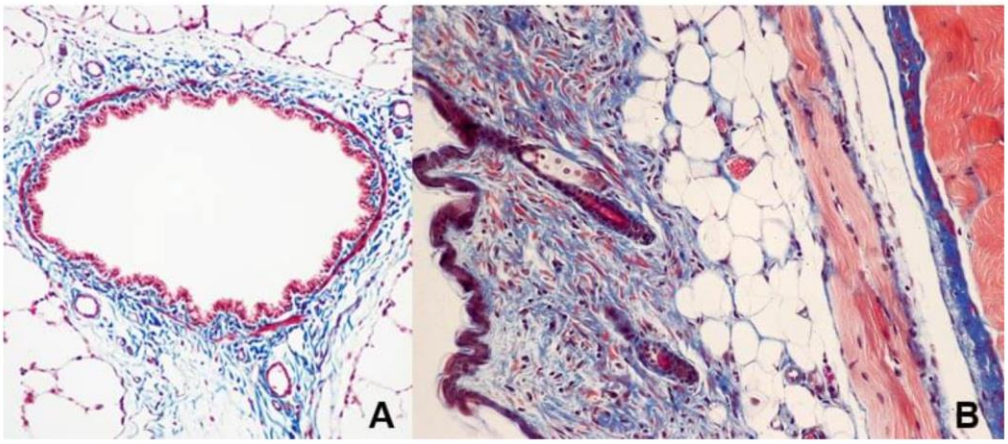

Figure 1.37: Figure A shows MT stain of a rat airway. Connective tissue is stained blue, nuclei are stained dark red/purple, and cytoplasm is stained red/pink. Figure B shows mouse skin stained with MT stain [106].

Elastin fibres in traditional histological staining are identified by Verhoeff Van Gieson staining, and was first described in 1889 by Ira Van Gieson to identify collagen fibres in neural tissue; and later modified by Frederick H. Verhoeff enabling the distinction between collagen and elastic fibres [107]. The initial stage is the Verhoeff stain component, using hematoxylin, iron (III) chloride and an iodine solution. Iron (III) chloride and iodine act as mordants and aid in the oxidation of hematoxylin to hematein, which is responsible for staining elastic elements. Elastin possesses a strong affinity for the hematoxylin-iron complex and thus, retains the stain longer than other tissue elements following decolourisation. Excess iron (III) chloride is used to differentiate the tissue; then sodium thiosulfate is used to remove excess iodine. The subsequent Van Gieson counterstain utilises picric acid and acid fuchsin to stain collagen and muscle fibres, producing contrast against the hematoxylin stain. The procedure results in elastic fibres and nuclei being stained black, collagen stained red and cytoplasmic elements stained yellow in light microscopy [Figure 1.38] [108].

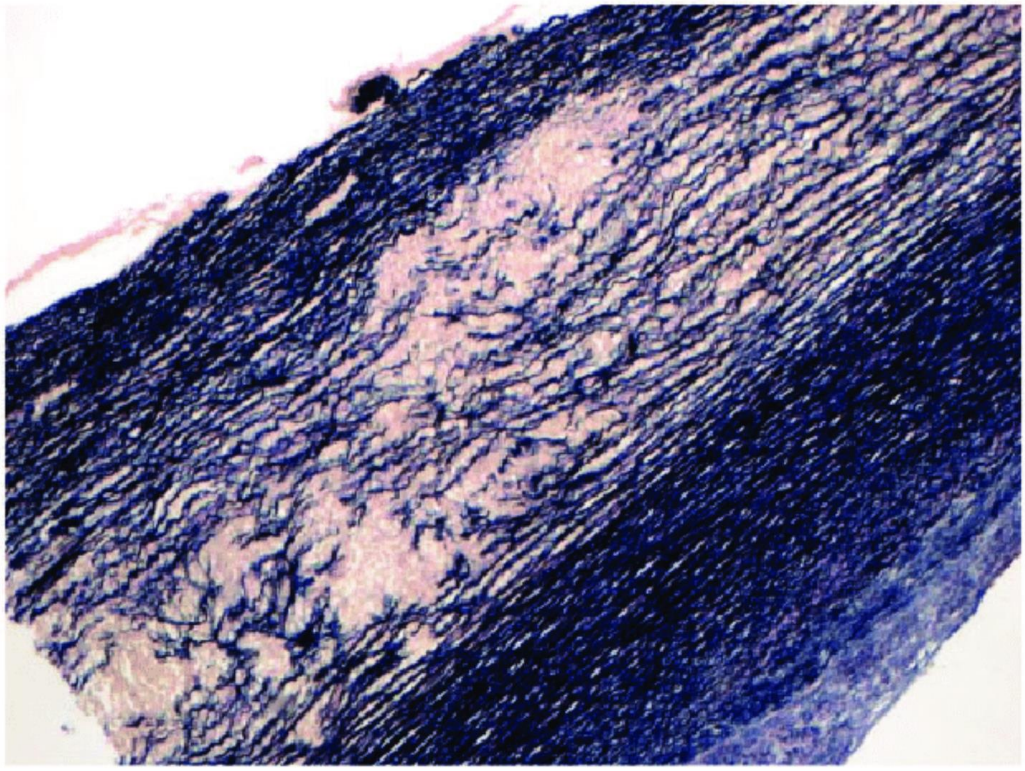

Figure 1.38: Aortic wall stained with Verhoeff Van Gieson stain, showing disruption of the elastic fibres within the elastic media [109].

The quantification of elastic fibres within the tissue is now determined commonly through Image J/Fiji imaging software.

1.6.1.2 Colour deconvolution analysis

In TAA's, elastin content is decreased compared with non-dissected controls and is hypothesised to be related to decreased expression of fibulin-5 who is known to be involved in elastogenesis [110]. Disrupted and irregular elastin have been observed in the medial layer of TAA's with either no elastin framework or severe elastic fibre fragmentation [111, 112]. Increasing fragmentation of elastic fibres has been found in dissecting aortas and aneurysms [113]. The elastin was found to be no different in content and concentration in regional analysis in dissected TAA and healthy controls [114]. The degree and quantification of fibrosis through the measurement of collagen in aortitis mouse models have been measured through CD [Figure 1.39] [115]. Similarly, collagen quantification was measured via CD for histological analysis of the aorta in different animal groups exposed to chronic hypoxic conditions, showing correlated results between mechanical and histological results [116].

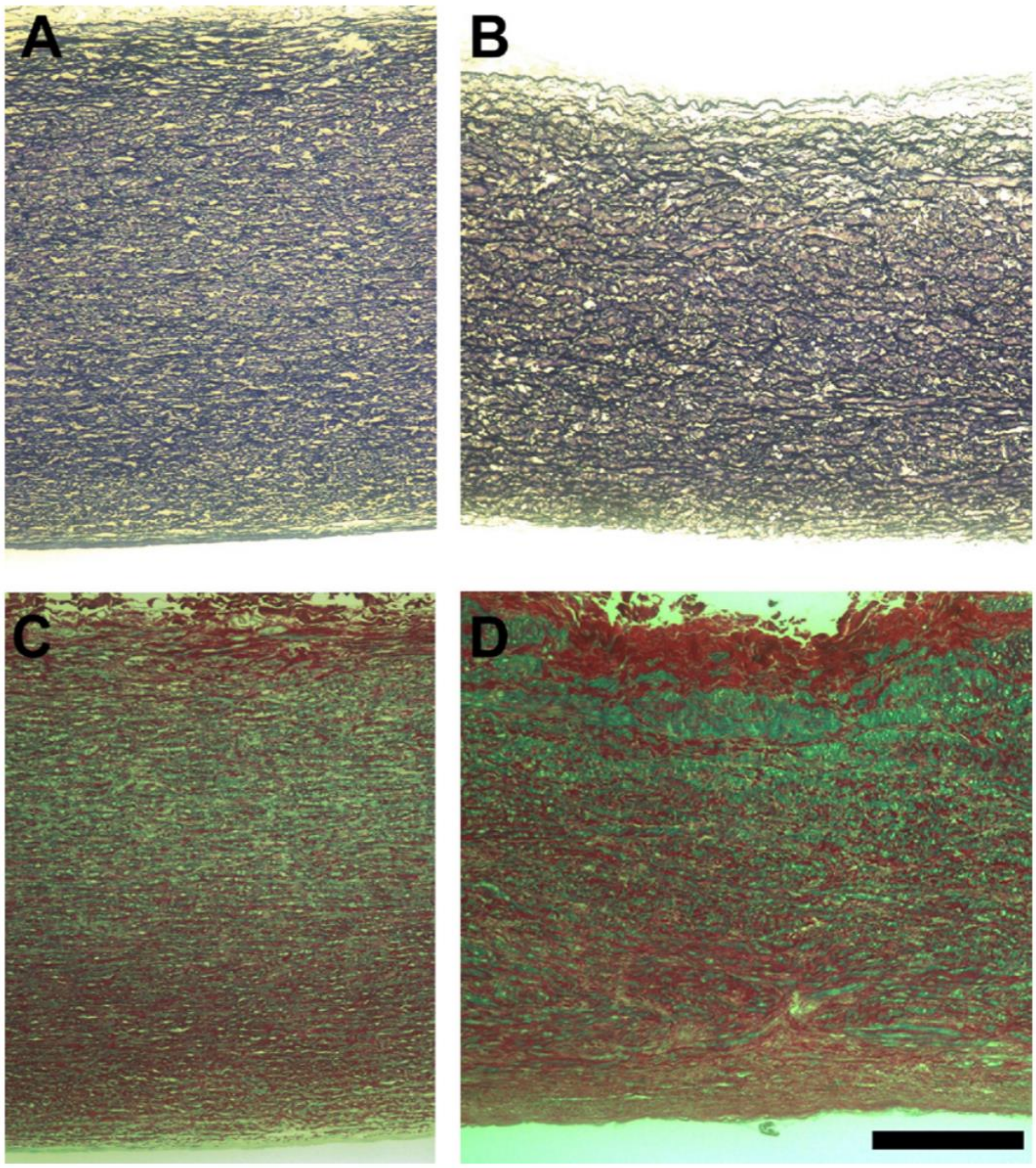

Figure 1.39: Histological specimens of the thoracic ascending aorta and aortic root sinuses. (A) shows elastin-stained ascending aorta, (B) shows elastin-stained aortic sinuses, (C) shows Sirius red-stained ascending aorta to highlight collagen fibres and (D) shows Sirius red-stained aortic sinus tissue to highlight collagen fibres. Image adapted from [115].

1.6.1.3 Immunohistochemistry (IHC)

The principle of IHC has existed since the 1930s, but it was not until 1941 that the first IHC study was reported. Immunohistochemistry is an important application of monoclonal as well as polyclonal antibodies to determine the tissue distribution of an antigen of interest in health and disease. While basic histologic examination of tissue is considered a useful and necessary component, IHC may provide a greater insight [117]. Immunohistochemistry requires the availability of tissue, which are processed into sections with a microtome and then incubated with an appropriate antibody. The site of antibody binding is visualised under an ordinary or fluorescent microscope by a marker such as fluorescent dye, enzyme, radioactive element, or colloidal gold, which is directly linked to the primary antibody or to an appropriate secondary antibody.

Quantitation of collagen types is difficult since total extraction is seldom achieved in biochemical analysis and their solubility depends on several properties including the degree of cross-linking between collagen molecules. Furthermore, in most tissues anatomical localisation of collagen types is not possible even when careful dissection techniques are coupled with the extraction procedure.

The isolation, purification, and immunochemical characterisation of collagen types has resulted in the development of well-defined antibodies to each collagen type, which can be used both for quantitation of extracted collagens and for identification of these types by immunohistological techniques. The latter methods permit precise anatomical localisation of collagen types in tissue sections. The antibodies employed are raised in animals, and isolated from antisera by immunoadsorption on immobilised antigens. Their specificity and potency are characterised by radioimmunoassay. There is a substantial cross-reactivity of bovine and human collagens and these antibodies have been used successfully in the study of human tissues [118]. More commonly, rabbit antibodies are used. Similarly, quantification and shape description of elastin fibres are achieved using IHC in a very specific way, without interference of other structures that may also be stained by standard histological techniques [118].

Patients with AAAs exhibit arterial dilation and altered matrix composition throughout the vasculature. Phenotypically, there is dissolution and fragmentation of collagen and elastin, which leads to expansion of the vessel wall that can no longer withhold the repetitive expansible forces of systolic contraction. These observations have strongly correlated with immunohistochemical findings of increased immunoreactivity to these components [119]. Immunohistochemistry analysis of collagen provides a precise analysis of both location and intensity of such antibodies [120].

1.6.1.4 Immunohistochemical analysis

Collagen is considered the most important component of the aortic wall and the amounts of collagen and collagen type ratios can change with ageing, sex hormone influence, and pathology. With age, the aortic wall becomes stiffer, with incremental increases in collagen content [28, 115, 120, 121].

The two main types of collagens found in the aorta are types I and III. They account for 80-90% of the total collagen present in the aorta. Types IV, V, VI and VIII can be also found in smaller amounts. In the normal aorta, fibrillar collagens (types I and III) are the major constituents of the intima, media, and adventitia layer. Types IV and V of collagen are situated in the endothelial and smooth muscle cell basement membranes, along with collagen types I and III [122]. Maurel and colleagues [123] stated that with age the quantity of collagen III decreased from the heart to the distal portion of the aorta, while other studies showed no change in collagen or elastin content with age [124, 125, 126, 127].

The presence of type III collagen in the aortic wall increases the flexibility of the collagen fibrils. Other studies demonstrate the integral role of type I collagen in the biomechanical and functional properties of the aorta. In the ascending aorta, types I, III, and IV constitute the intima and media layers.

1.6.1.5 Immunohistochemical analysis of the pathological aorta

In humans, higher levels of collagens type I, III, and collagen cross-linking are reported in aneurysmal aortas [76] and are thought to enhance arterial stiffness and susceptibility to dissection and rupture. On the other hand, decreased collagen content and cross-linking can weaken the aortic wall, leading to aneurysm formation and/or aortic dissection. The disparity in collagen content might reflect different phases of aortic remodelling, with fibrosis occurring at the late phase of inflammation during vessel repair. This also highlights the importance of sustaining a balance in collagen content for optimal aortic structure and function [128].

In control thoracic ascending aorta and normal histological samples of ascending aorta dissection, type IV collagen were seen between the subintimal basement membrane and the media, and in the basement membrane of the adventitia [129]. In cases of ascending aorta dissection with CMD or medionecrosis there were often areas of missing collagen resulting in a disorganised structure. Collagen staining of types I and III was more intense in cases of ascending aorta dissection than in controls and were characterised by thick longitudinal sheets or bundles in the media which were larger than type IV [124, 130]. Collagen proportional changes have also been reported in thoracic ascending aorta dissection, with reduced collagen percentage in the inner half of the media (12% +/- 33) and in the outer half of the media (12% +/- 19).

The ratio of type I to type III collagen was also suggested to be important in aortic aneurysms. Menashi and colleagues [131] estimated that this ratio did not vary significantly from 2:1 in both control and aortic aneurysms groups. Rizzo and colleagues estimated that collagen type I accounted for 74% ± 4% of aneurysm and 73% ± 4% of control. Collagen type III accounted for 26% ± 4% of aneurysm and 27% ± 4% of control [132].

1.6.1.6 Immunohistochemical analysis of the pathological aortic root

The SOV within the aortic root has been the focus of the limited available studies, and varied IHC findings have been reported.

There have been reports that the collagen structure alters specifically and significantly: collagens type I and III decrease, while collagens alpha-1 (XI) and V increase [133]. Others opposed this view stating that the amount of collagen in thoracic root aneurysms increase [134], or that the collagen content did not differ notably between aneurysmal and control sinuses [135].

It is agreed that fragmentation of collagen and disconnection from the network structure would impair normal aortic root function, regardless of the amount of collagen or quantification measure [136].

1.6.2 Animal models

1.6.2.1 Animal models utilising cardiopulmonary bypass

Several pig models have been produced that have aimed to reproduce normal circulatory blood flow however no animal model has replicated high aortic pressures beyond that of which is possible in human subjects to truly test the biomechanical limits of the aortic root and ascending aorta. This further emphasised the unique nature of our study design.

The literature suggests that the pig aortic model is a suitable surrogate for the human aorta since they are structurally the same and thus an appropriate model to study the pathophysiology of rupture.

See Table 4.1 (Chapter 4) for a review of animal models utilising CPB.

1.6.2.2 Histopathological analysis in pig studies

Despite the structural similarities and appropriateness of surrogate testing, protein quantification in pig tissue and aorta is scarce in the literature. A study in 1985 from Davidson and colleagues [137]

aimed to determine this in newborn pigs. Relative collagen and elastin syntheses, as a per cent of total protein synthesis, were determined in four separate experiments. Elastin synthesis decreased from about 16.4% in the thoracic aorta to 1.6% of total protein synthesis in the abdominal aorta. Collagen synthesis showed the opposite trend, increasing to 12% of total protein synthesis, although collagen synthesis was still a significant fraction (5-8%) of total protein synthesis in the upper thoracic tissue [137]. Collagen composition was reported as higher in the proximal inner and outer regions of our samples on average across all specimens. Elastin composition was also recorded highest in the inner regions across proximal, middle, and distal aortic regions.

By identifying a clinical and histological difference between the aortic root and ascending aorta the somewhat current generalised surgical management of these areas have the potential to evolve into focused management of aortic root aneurysms, and ascending aorta aneurysms as independent structures, and independent pathologies.

1.6.3 Outcome measures

1.6.3.1 Patient related outcome measures following aortic valve replacement

There is a transition in recording the factors of medical and surgical outcomes that matter to patients instead of technical aspects or imaging parameters (e.g., paravalvular leaks). Health status is the impact of disease on patient function as reported by the patient. More specifically, health status can be defined as the range of manifestation of disease in each patient including symptoms, functional limitation, and quality of life, in which quality of life is the discrepancy between actual and desired function (Figure 1.40). There is often a large discrepancy between physician-rated and patient-rated symptom burden and functional limitation and traditional clinical testing is limited. For care to become more patient-centred, we need to use standardised patient surveys to measure the complete spectrum of health status [138]. These surveys can be in the form of Patient related outcome measures (PROMS).

Patient related outcome measures are instruments used to measure patient-related outcomes (PRO). A PRO is directly reported by the patient without interpretation of the patient's response by a clinician or anyone else and pertains to the patient's health, quality of life, or functional status associated with health care or treatment [138].

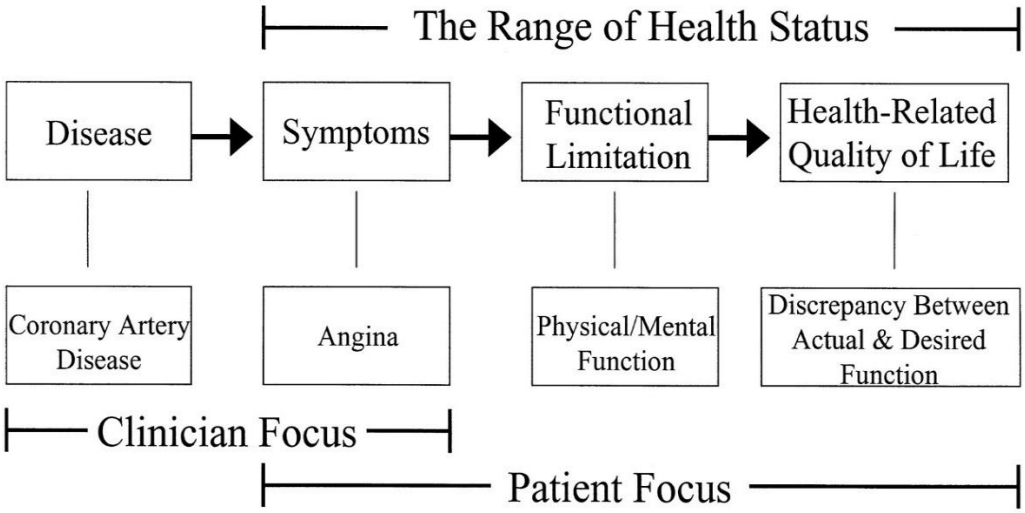

Figure 1.40: The range of health status: symptoms, function, and quality of life [138].

PROMS are used for the following broad purposes:

- Clinician and consumer decision making - enhancing individual clinician-patient interactions and care; and enable tailoring of services to provide the care that the patients need and want.
- Quality improvement – comparing the effects of different treatments, and for understanding unwarranted clinical variation.
- Population-level surveillance and planning, and informing policy and funding models.

Internationally, such routine and consistent measurement is already embedded in the health systems of several Organisation for Economic Co-operation and Development (OECD) countries. Countries most advanced in implementing PROMs at a national or jurisdictional level are England (referred to as the NHS), the Netherlands, Sweden and the United States, and an increasing interest in a national approach in Canada. The implementation of a standardised approach aims to support the systematic collection, analysis, and timely reporting of PROMs to clinicians so that they can provide the best care to patients. Results would be available during clinical encounters to enable patients and clinicians to make decisions together. Aggregated data would also be available to use as a measure of service quality, and at a system level to drive excellence and innovation and inform value-based healthcare models.

Quality-of-life analysis in AV surgery patients was first published in 1997 and 2000 with a retrospective analysis of outcomes in octogenarians (patients 70 years and older, and patients 80 years and older respectively) receiving a SAVR [139, 140]. Quality of life was determined using the Short-Form 36 (SF-36) tool which showed results comparable with aged-matched population norms, except for mental health. Patient related outcome measures were first applied in the areas of heart failure [141] and later to heart valve surgery in 2016 [142] to assess outcomes in a more

detailed way. This review of the use of PROMS in heart valve surgery showed that various PRO tools were valuable in assessing a patient's quality of life before and after cardiac surgery. The earlier reports on outcomes following TAVR were on early experiences in the STS/ACC TVT registry [143]. Quality of life was extracted from collected registry data however the use of questionnaires was not included. The Partner trials have compared outcomes between SAVR and TAVR in low, intermediate, and high surgical risk groups since 2012. The Partner 1 trial in 2012 reported on PROMS in TAVR and SAVR patients in the areas of heart failure (KCCQ), quality of life (EQ5D) and generalised health status (SF12). Quality of life and health status was maintained at 12 months follow up [144]. The Partner 2 trial in 2016 assessed baseline heath status using Kansas City Cardiomyopathy (KCCQ) SF 36, and EQ 5D questionnaires. This was reported over a 1-2-year follow-up [145]; and the Partner 3 trial in 2019 assessed functional status and quality of life at 30 days and 1 year using a 6-minute walk test, and KCCQ score. Conclusions were that TAVR had rapid improvements in symptoms of failure, 6-minite walk test distance, and KCCQ score compared to surgery [146]. The partner trials focus on quality of life and functional status as secondary endpoints with only the Partner 2 trial referring towards a specific quality of life questionnaire in the use of the EQ5D. In these large trials over the last 10 years which have defined clinical practice, there has been little analysis on quality of life and angina, and no reference towards depression and frailty as primary or secondary endpoints.

The most common way of measuring a PRO is using standardised, validated questionnaires. These questionnaires ask the patient to rate their health by responding to a series of items, which are then combined to represent an underlying construct such as pain, symptom severity, function, or quality of life. Generally, the analysis of PROMS focuses on the change in scores following an intervention such as surgery or medical treatment course [139]. Development of a PRO instrument involves five key steps as outlined in Figure 1.41 [140].

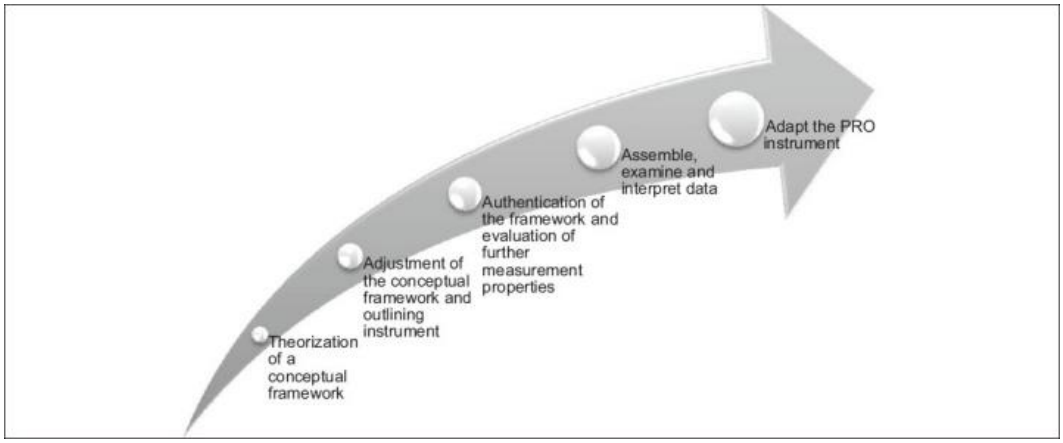

Figure 1.41: Development of a PRO instrument. Image adapted from [140].

Patient related outcome measures can be used for routine data collection, and such a modality is utilised in the South Australia State-wide patient reported measures program. Selection should

always occur with consideration of specific objectives, samples, treatments, and available resources. A suggested principle includes: (1) always considering PROMs early in the study design process; (2) choose a primary PROM that is as proximal to the specific pathology or target intervention; (3) identify candidate PROMs primarily on the grounds of scaling and content; (4) appraise the reliability, validity and 'track records' of candidate PROMS in studies similar to that planned; (5) look ahead to practical concerns; and (6) take a minimalist approach to ad hoc items (4).

1.6.3.2 Seattle angina questionnaire (SAQ-7) in measuring anginal outcomes

Angina pectoris is a commonly reported symptom of aortic stenosis with or without the presence of coronary artery disease (CAD) [147]. The presence of angina in the absence of CAD has been described in several studies from as early as 1951.

Author	Year	Findings
Lewes D [148]	1951	• Reviewed the clinical findings in 22 cases with AS • Anginal pains followed rapidly progressing left ventricular failure • 3 cases gave a clear history of anginal pains
Mitchell et al. [149]	1954	• Case series of the clinical symptoms of AS involving 533 patients • 159 patients (29.8%) of patients reported having angina • 65% of the patients with angina were males • The appearance of the coronary arteries was described in only 11 of the cases with angina and 11 of these cases had moderate to advanced CAD
Wood P [150]	1958	• Analysed a series of 250 cases of AS • Angina pectoris was present in 70% of cases
Baker and Somerville [151]	1959	• No online data available
Basta et al. [152]	1975	• Reviewed 88 patients with severe aortic stenosis over a period of 5 years from 1968 to 1973 • 51 patients reported angina pectoris • Significant CAD was found in 24% of patients with AS and 20% of patients with AR

Table 1.2: Early studies reporting on the presence of angina pectoris in AS

More recent studies have focused on testing the occurrence of angina in patients with non-obstructed coronary artery disease [153, 154, 155] and the results of these studies are as follows.

Author	Year	Findings
Lumley et al [153]	2016	• Measurement of intracoronary pressure and flow measures in 22 patients with severe AS • Concluded that ischemia in AS is not related to microvascular disease, rather driven by abnormal cardiac-coronary coupling
Gould et al [154]	2016	• Reviewed the current literature on angina in patients with AS • Concluded that discussed studies may have discovered normal microvascular function in AS due to patient cohort having low prevalence of comorbidities
Rajappam et al [155]	2003	• Studied 22 patients before and after AVR • Areas measured included myocardial blood flow, left ventricular mass regression (LVM), and aortic valve area (AVA) • Changes in microcirculation after AVR in patients with AS are not directly related to LVM regression

Table 1.3: Studies focusing on the investigation of obstructed coronary arteries in those undergoing AVR.

The Seattle Angina Questionnaire as a formal functional measure of coronary artery disease was developed in 1995 [156]. It measures five clinically important dimensions of health in patients with coronary artery disease including: physical limitation, anginal stability, anginal frequency, treatment satisfaction, and disease perception.

1.6.3.3 EQ-5D questionnaire in measuring quality of life outcomes

The EQ-5D was first introduced in 1990 by the EuroQol Group initially formed in 1987 in Europe with the aim of developing an instrument that is standardised and can be used as a complement for existing health related quality of life measures. It asks patients to report on the five dimensions of their health: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension is scored on a three-point scale, where 1 = no problems, 2 = some problems and 3 = extreme problems. All possible combinations of scores across the five dimensions can be used to produce health state values [142]. The EQ-5D questionnaire is a generic questionnaire and has been used widely across a variety of disciplines. In the field of Cardiothoracic Surgery, its utility is less commonly utilised. The EQ-5D has been used in several studies comparing the use of various health questionnaires in reporting outcomes in patients post coronary bypass grafting (CABG) and AS surgery. The construct validity of the questionnaire was reported in 2005 in acute coronary syndrome (ACS) patients. Compared to the SF-8, patients were followed up over a 3-year period. The study demonstrated clear construct validity of the EQ-5D in a population-based sample where 40.3% of the study sample of 1217 patients responded [143]. When compared to its more extensive

15 question version (EQ-15D) in a prospective cohort study, the two versions did not appear to be interchangeable when patient-centred outcomes are assessed. The EQ-5D was deemed to have better discriminative power and known-group validity whereas the 15D was more sensitive to change over time [157]. A single-centre study compared TAVR and SAVR health-related outcomes at baseline, one month postoperatively, and one-year following surgery via telephone questionnaire. A significant improvement in QOL was seen in both groups. Assessing patients QOL through the EQ5D was deemed valuable in helping clinicians make informed decisions about the best possible treatment approaches for their patients [142]. This was further supported in multiple studies reporting on TAVR clinical outcomes after 1 year and 4 years respectively [158, 159].

1.6.3.4 PHQ-9 questionnaire in measuring depression outcomes

The PHQ-9 depression questionnaire was developed by Spitzer and Colleagues in 1999 as a brief self-report inventory derived from the PRIME-MD clinical interview to measure the nine core DSM-IV diagnostic symptoms associated with a depressive episode. It was validated and widely used as a brief diagnostic and severity measure [160], but was not immediately validated as an outcome measure for depression. The sensitivity to change in the PHQ-9 in three groups of medical outpatients with major depressive disorder was determined. They found that the changes in PHQ-9 score corresponded with changes in depression diagnostic status over time, providing preliminary evidence that the PHQ-9 can be used for longitudinal as well as for cross-sectional studies. It was concluded that the PHQ-9 can detect depression outcome and changes over time. A detailed metanalysis of studies using the PHQ-9 as a measure of depression screening found a low sensitivity of the questionnaire in a high heterogeneity between studies [161]. Further studies found that the PHQ-9 was a valuable screening measure rather than a diagnostic tool (162, 163, 164].

1.6.3.5 Essential frailty toolset (EFT) in measuring frailty outcomes

The Essential Frailty Toolset (EFT) scores from 0 (least frail) to 5 (most frail) based on 4 items: pre-procedural anaemia, hypoalbuminemia, lower extremity muscle weakness defined as a time of greater than 15 seconds or inability to complete five sit-to-stand repetitions without using arms, and cognitive impairment defined as a score of less than 24 on the Mini-Mental State Examination [165]. In a prospective cohort study of over 1000 patients undergoing SAVR and TAVR, a comparison between 7 frailty scales occurred. The frailty scales tested were Fried, Fried+, SPPB, Rockwood, Bern, Columbia, and the EFT. The EFT outperformed the other frailty scales to identify vulnerable older adults who are at higher risk of poor outcomes after SAVR and TAVR.

A scoping literature review revealed the following questionnaires have been used in a variety of studies to determine outcome measures in the areas of angina, depression, quality of life and frailty.

No studies have combined their use in the measure of patient outcomes following SAVR and TAVR [Table 1.4].

Black et al. 2014 [166]	**EQ5D**	• Measured patient related outcomes and experience in elective surgery for knee replacement, hip replacement and groin hernia's using EQ-5D questionnaire which explores mobility, self-care, usual activities, pain/discomfort, and anxiety/depression • They reported a positive association between patients' experiences and their reports of effectiveness for all three procedures. A positive relationship was also apparent when effectiveness was based on patients' reports of the extent of improvement in their health. Patient reported experience was also associated with safety
Varagunam et al. 2015 [167]	**EQ5D**	• Measured patient related outcomes in elective surgery considering hospital volume and consultant load using an EQ-5D 3L questionnaire • There was no significant association between hospital volume and outcome of surgery for all 3 procedures assessed, but higher consultant volume in hip replacement surgery was association with a greater gain in functional status
Straatman et al. 2016 [168]	**EQ5D**	• Measured outcomes in postoperative gastrectomy using ten different PROM questionnaires: including SF 12 and EQ 5D types. A questionnaire with a more general module to assess overall QOL along with a disease specific module for assessment or quality of life was recommended
Holmes et al. 2016 [169]	**EQ5D**	• Measured patient related outcomes in heart valve surgery patients using EQ 5D and disease specific Minnesota heart failure questionnaire which measures physical, emotional and socioeconomical outcomes • Both EQ5D and MLHFQ registered significant improvements in patients' health
Mason et al. 2014 [170]	**EQ5D**	• Investigated the use of PROMS in emergency surgical admissions. The EQ5D, SF12 and GIQLI were chosen because they are the most used and validated PROMs in studies investigating outcomes in non-trauma emergency surgery
Abah et al. 2015 [171]	**EQ5D**	• Measured patient related outcomes in cardiac surgery patients using the SF 36, EQ 5D, Hospital anxiety and depression scale (HADS) and MLFHQ questionnaires • Quality of life was reduced in 8-19% of patients following cardiac surgery. Majority of patients indicated an improvement in postoperative quality of life
Partner 2 trial 2016 [145]	**EQ5D**	• Assessed baseline heath status using Kansas City Cardiomyopathy (KCCQ) SF 36, and EQ 5D questionnaires. This was reported over a 1-2-year follow-up • Limitations in this trial include, transthoracic cohort was low (24%) and therefore comparisons between TAVR and SAVR are underpowered
Stenman et al. 2019 [172]	**PHQ-9**	• Reported on patients having CABG as a screening tool

		• 64% response rate revealing 15% scoring >10 indicating severe depression
Kroenke et al. 2002 [173]	**PHQ-9**	• A 9-question version of the validated PRIME-MD instrument used in evaluation of mental health disorders was developed due to the unacceptable high false positive rate in the PHQ-2 • The PHQ-9 had exclusive focus on the 9 diagnostic criteria for the DSM-IV depressive disorders and therefore deemed attractive in making diagnoses and assessing severity of depressive disorders
Lowe et al. 2004 [161]	**PHQ-9**	• Determined the use of the PHQ as an outcome measure of depression in three groups of whose depression improved, remained unchanged or deteriorated • PHQ-9 scores differed significantly between the three depression outcome groups • Demonstrated the ability of the PHQ-9 to detect depression over time
Arroll et al. 2010 [174]	**PHQ-9**	• Compared the PHQ-2 and PHQ-9 as a validation tool in unipolar depression • Enrolled 2642 patients • The PHQ-2 had poor specificity in detecting major depression and the PHQ-9 had similar sensitivities but superior specificities in detecting cases of major depression
Manea et al. 2012 [162]	**PHQ-9**	• Metanalysis to determine the optimal cut-off score in the diagnosis of depression in the PHQ-9 questionnaire • 18 validated studies were identified in various clinical settings • The PHQ-9 was found to have acceptable diagnostic properties for detecting major depressive disorder for cut-off scores between 8 and 11
Afilalo et al. 2017 [165]	**EFT**	• Compared 7 frailty scales to predict outcomes following SAVR or TAVR • Outcomes of interest was all cause mortality and disability 1 year after the procedure • The EFT outperformed all other frailty scales is recommended for use in this setting
Saji et al. 2020 [175]	**EFT**	• Assessed the validity if the EFT as a predictor of all-cause mortality following TAVR • 176 patients with severe AS were enrolled • The modified EFT score was independently associated with all-cause mortality • The modified EFT score had excellent predictive performance for all-cause mortality at 1 year
Chan et al. 2014 [176]	**SAQ-7**	• Validation study on the shortened version of the SAQ from 19 items to 7 • The SAQ-7 demonstrated good construct validity, was reasonability reproducible patients with stable CAD and had good responsiveness in patients post PCI • SAQ-7 was predictive of 1-year mortality and re-admission
Spertus et al. 1995 [156]	**SAQ**	• Validation of the SAQ 19 item questionnaire • Cross sectional analysis across 4 groups of patients over a 3-month interval • The questionnaire was sensitive to dramatic and subtle clinical change

		• Determined that the SAQ was a valid and reliable instrument in the measure of outcomes in coronary artery disease
Patel et al. 2018 [177]	**SAQ**	• To determine the validity of health status in a male and female population with ischemic heart disease • Tested 5 SAQ subdomains separately in men and women • SAQ demonstrated similar results for men and women with coronary artery disease

Table 1.4: Earlier studies reporting on PROMs incorporating the 3 questionnaires used in this study (PHQ9, EQ5D, EFT, and SAQ-7) in surgical patients.

The following studies report on the patient related outcome measures in cardiac surgery, with a specific focus on SAVR and TAVR [Table 1.5].

Stanska et al (2018) [159]	**EQ5D**	• Evaluated short term QOL changes in patients undergoing SAVR and TAVR • QOL was measures at baseline, 1 month and 1-year using EQ5D • A significant improvement in QOL was observed in all groups • SAVR patients reported lower health status compared to TAVR
Kaier et al. 2016 [178]	**EQ5D**	• Evaluated QOL over a 2-year period in SAVR, TAVR, and medically managed patients with AS • QOL measures decreased slightly over time
Lange et al. 2016 [158]	**EQ5D**	Evaluated QOL in TAVR patients in the GARY registry using EQ5D at baseline and 1 year • TAVR treatment led to improvements in QOL especially in terms of mobility and usual activities
Ronde-Tillmans et al. 2018 [179]	**EQ5D**	• Single centre study evaluated QOL outcomes over 4 years using EQ5D and SF36 questionnaires • All patients showed a satisfactory improvement in functional class (NYHA) and QOL despite age and comorbidities • Could not perform an age-matched or co-morbidity matched comparison
McIntosh et al. 2018 [180]	**EQ5D**	• A retrospective analysis on TAVR outcomes using QOL questionnaires • Performed at 30-days and 1-year post operatively • TAVR lead to significant QOL improvements, including cognition and frailty indices
Stenman et al. 2019 [172]	**PHQ 9**	• Prospective cohort study investigating 1-year longitudinal outcomes of depression screening in cardiac surgery patients • Depression at baseline was twice as common as in men, 10% who screened negative at baseline, were positive after 1 year, women were more negative than men after 1 year in PHQ-9 screening
Horne et al. 2016 [181]	**PHQ-9**	• Prospective cohort investigating 6-month outcomes in the influence of physical activity on mood following cardiac surgery • At each time interval of questionnaire patients were labelled as depressed or not depressed • Patients who were depressed median PHQ-9 score continued to decrease from baseline to discharge
Tully et al. 2016 [182]	**PHQ-9**	• Prospective cohort investigating 6-month longitudinal outcomes of routine depression screening in cardiac surgery patients • PHQ9 only used at 30-days post-surgery with SF12 being used at 6 months • Depression screen positive group had higher risk of depressed mood and poorer QOL in all domains
Afilalo et al. 2017 [165]	**EFT**	• Compared to a few other frailty toolsets over 14 centres and 3 countries, the EFT was considered the strongest predictor of worsening disability at 1-year
Skaar et al. 2019 [183]	**EFT**	• Observational study over a 4-year period in patients undergoing TAVR

		• Missed several important parameters including time taken to rise from chair (item 1) and serum albumin levels (item 4) • Compared the GA frailty score to the EFT • Concluded that the lack of data used in calculating the EFT reduces its precision • The GA frailty scale > or equal to 4 had significantly higher 2-year mortality
Drudi et al. 2018 [184]	**EFT**	• Reported on site outcomes in TAVR patients with preprocedural EFT scores to determine frailty • Non-femoral access is associated with greater risk of 30-day and 12-month mortality after TAVR particularly in frail patients
Piankova et al. 2020 [185]	**EFT**	• Systematic review of frailty scale use on outcomes following TAVR • Frailty was significantly associated with short term <6-month mortality, midterm mortality 6-36 months, procedural complications (bleeding, transfusions, delirium), and with worsening disability and poor outcome • Author's recommendation was use of the EFT screening tool and more focused frailty assessment if problematic domains are identified
Schroter et al. 2006 [186]	**SAQ**	• Compared 3 validated quality of life instruments to compare outcomes in patients undergoing CABG or percutaneous angioplasty • Testing administered prior to procedure and 3 months post procedure • SAQ was most responsive in terms of physical functioning • SAQ and CROQ (coronary revascularization outcome questionnaire) were equally responsive as an overall outcome instrument
Hersovici et al. 2018 [187]	**SAQ-7**	• SAQ-7 was used in the WISE-CVD trial to assess the results of SAQ in women with signs and symptoms of ischemia but no obstructive coronary disease • SAQ-7 showed association with angina hospitalisation • SAQ-7 appeared to be a good predictor of angina hospitalisation in women and may be a useful predictor of women with non-obstructive coronary disease
Dougherty et al. 1998 [188]	**SAQ**	• Used as a comparison to other quality of life tools to determine outcomes in patients with stable angina • 107 patients tested in a randomized trial • SAQ detected changes in heart disease over time and demonstrated acceptable re-test reliability when tested over a 2-week interval

Table 1.5: Literature review of studies reporting on PROMS outcomes in the areas of depression, quality of life, frailty, and angina

The evolving transcatheter techniques in the management of aortic valve disease are an inevitable change that we are witnessing year to year, and there is no argument as to the value in its application to certain cases of aortic valve implantation. However, in the short-term review of clinical outcomes to date there has been a gap in discussion on the clinical morbidity that occurs

over the mid to long term, and the clinical outcomes that affects patients' quality of life. By identifying the underreported clinical outcomes, we have the potential to manage patients with aortic valve disease in a more complete and best way for the long term.

1.7 Guidelines and Treatment

1.7.1 Therapies

1.7.1.1 Aortic aneurysm

Patients with asymptomatic TAA should be followed for the development of signs and symptoms that may be associated with aneurysm progression. The surveillance schedule is based upon the aetiology, site, and diameter of the aneurysm at presentation, and expansion rates identified at follow-up. Ideally, serial CT or magnetic resonance (MR) angiography studies should be performed using the same imaging technique at the same centre. For patients with asymptomatic TAA who are being conservatively managed, control of hypertension is recommended to limit further aortic expansion [189].

Symptoms such as chest pain in a patient with TAA (known or unknown) can represent rapid aneurysm expansion or be due to a variety of life-threatening complications, including aortic dissection, acute aortic regurgitation, aortic leakage, or overt aortic rupture. Patients who develop symptoms attributable to TAA should undergo urgent repair (open surgical, endovascular), provided the risk for repair is not prohibitive.

Elective repair of asymptomatic TAA is not undertaken until the risk of rupture or other complications exceeds the risks associated with repair. Selection is based on diameter, location, expansion rate, and patient comorbidities, considering the presence of underlying contributing aetiologies. The most important factor determining the risk for TAA complications is the diameter of the aneurysm. Patients with a genetically influenced TAA, aortic diameter thresholds are lower. A decision for repair also needs to consider the diameter of the aortic root, as well as coronary artery disease and AV pathology that may require surgical intervention at the same time.

1.7.1.2 Aortic stenosis

Two-dimensional (2D) echocardiography is the primary diagnostic tool in valvular heart disease. Aortic stenosis is characterised by decreased mobility of the aortic valve leaflets, the presence of calcification, and flow acceleration across the valve. It relies on three parameters, namely the peak velocity (PVel), the mean pressure gradient (MPG) and the aortic valve area (AVA). Severe AS being defined by a peak velocity >4 m/sec, an MPG >40 mmHg and an AVA <1 cm^2 (Table 1.6) [190].

Three-dimensional (3D) echocardiography may provide additional information on valve morphology (bicuspid or tricuspid) and location of calcification, which is helpful for procedure planning if the patient is referred for SAVR or TAVR.

Four-dimensional (4D) computed tomography (CT) is increasingly used for determination of specific valve anatomy, particularly in planning transcatheter valve interventions. Four-dimensional CT and magnetic resonance imaging (MRI) can provide aortic valve area data that correlate well with echocardiography and may be valuable in patients with poor echo windows, when additional anatomic data are desirable, for example, ascending aortic diameter or arch calcification [191].

Echo parameters	**Sclerosis**	**Mild AS**	**Moderate AS**	**Severe AS**
Peak velocity, m/sec	<2.5	2.5-3	3-4	>4
Mean gradient, mmHg	Normal	<20	20-40	40
AVA, cm2	Normal	>or equal to 1.5	1-1.5	<1cm^2
Calcium scoring, AU				Male 2,065 Female 1,275

Table 1.6: AS grades of severity as assessed using echocardiography and computed tomography (calcium scoring) [190].

Surgical aortic valve replacement and TAVR are the mainstays of treatment of severe calcific AS, as they improve symptoms and prolong survival.

For patients with severe calcific native AS with an indication for intervention, a choice is made between SAVR and TAVR or palliative medical therapy based upon estimated surgical risk and other factors.

1.7.1.3 Aortic stenosis versus aortic regurgitation

There are two primary pathologies of the aortic valve, AS discussed, and aortic regurgitation (AR) or insufficiency. Aortic insufficiency occurs due to inadequate closure of the AV during diastole leading to retrograde blood flow from the aorta into the left ventricle. The consequences of this are an increase in left ventricular end-diastolic volume and wall stress [192].

Aortic regurgitation leads to retrograde flow of blood from the aorta into the left ventricle, causing an increased left ventricular volume, and dilation of the chamber. This results in an increase in

cardiac output. With persistent regurgitation, this increase in cardiac output leads to distention and increased pressure in peripheral arteries, causing increased peripheral systolic pressure. Worsening regurgitation results and causes a decrease in peripheral systolic pressure and, in severe disease, cardiovascular collapse [192].

Valve replacement for the treatment of AR is indicated for:

- symptomatic patients with severe AR regardless of LV systolic function
- asymptomatic patients with chronic severe AR and evidence of LV systolic dysfunction
- patients with severe AR while undergoing cardiac surgery for any other indication

Valve replacement is reasonable for:

- asymptomatic severe AR patients and normal LV systolic function but severe dilation of the left ventricle
- moderate AR patients who are undergoing other cardiac surgery
- asymptomatic patients with severe AR and normal LV systolic function but with evidence of progressive severe LV dilation if the surgical risk is low

Patients, whom life expectancy after the replacement is less than one year and/or quality of life is not expected to improve, would not be candidates for valve replacement. However, if life expectancy is greater than one year, and predictions include improvement in the quality of life, there are two methods for AV replacement, surgical or transcatheter [192].

Transcatheter aortic valve replacement is not indicated for aortic regurgitation. The main reason for this is that the noncalcified aortic valve lacks fluoroscopic landmarks and an anchor site for prosthesis, which tends to increase the risk of prosthesis dislocation in TAVR. In addition, absent or minimal calcification of aortic valve induced insufficient anchoring results in prosthesis dislodgement, which can lead to poor prognosis [193]. The lack of calcium, the increased stroke volume secondary to severe AR and the presence of aortic root dilatation makes device positioning and deployment very difficult and there is a predisposition to embolisation or malposition of the prosthesis with subsequent moderate to severe post-procedural AR (associated with worst clinical outcomes). Valve migration can occur to the aorta or deep into the LV up to several hours after implantation [193]. In a recent study by Alharbi and colleagues in 2020, off label indication of TAVR for AR versus SAVR resulted in significantly more cardiopulmonary resuscitations and permanent pacemaker (PPM) placements in the TAVR group [194].

The new 2020 AHA guidelines state that TAVR for isolated chronic AR is challenging because of dilation of the aortic annulus and aortic root and, in many patients, lack of sufficient leaflet calcification. Risks of TAVR for treatment of AR include transcatheter valve migration and significant paravalvular leak. Therefore, TAVR is rarely feasible, and then only in carefully selected

patients with severe AR and HF who have a prohibitive surgical risk and in whom valvular calcification and annular size are appropriate for a transcatheter approach [195].

New dedicated devices are being designed and those available are evolving to transfemoral applications. Applications at this stage are for inoperable severe AR because it offers a better prognosis than optimal medical treatment [194]. Transcatheter AVR is not yet the standard for treatment of AR, but is likely to be more established with time. Until then, SAVR remains the gold standard for treatment of a regurgitant aortic valve.

` 1.7.2 Guidelines in the management of aortic pathology

1.7.2.1 Ascending aorta

The surgical guidelines of the American Heart Association, Society of Thoracic Surgeons, American Association for Thoracic Surgery, and European Society of Cardiology recommend pre-emptive repair of ascending aorta aneurysms at a diameter of 5.5 cm and 5.0 cm for patients with connective tissue aortopathies whose behaviour dictates a more aggressive approach and earlier intervention. The cut-off value of 5.5 cm corresponds to a steep rise in the respective risk curve [47].

Surgery should be performed in patients with MFS, who have a maximal aortic diameter ≥50 mm. A lower threshold of 45 mm can be considered in patients with additional risk factors, including family history of dissection, size increase of>3 mm/year (in repeated examinations using the same technique and confirmed by another technique), severe AR, or desire for pregnancy. Patients with Marfanoid manifestations due to connective tissue disease, without complete Marfan criteria, should be treated as Marfan patients. Earlier interventions have been proposed for aortic diameters 42 mm in patients with LDS [17].

However, the underlying evidence is self-contradictory, and the Task Force chose not to recommend a different threshold for MFS [17]. Patients with EDS are exposed to a high risk of aortic complications, but no data are available to propose a specific threshold for intervention.

Surgery should be performed in patients with a BAV, who have a maximal aortic diameter ≥55 mm; these face a lower risk of complications than in MFS [189]. Similarly, as above, a lower threshold of 50 mm can be considered in patients with additional risk factors, such as family history, systemic hypertension, coarctation of the aorta, or increase in aortic diameter >3 mm/year, and according to age, body size, comorbidities, and type of surgery. Regardless of aetiology, surgery should be performed in patients who have a maximal aortic diameter ≥55 mm. In borderline cases, the individual and family history, patient age, and the anticipated risk of the procedure should be taken into consideration.

For patients who have an indication for surgery on the aortic valve, lower thresholds can be used for concomitant aortic replacement (≥45 mm) depending on age, body size, aetiology of valvular disease, and intraoperative shape and thickness of the ascending aorta. The choice between a total replacement of the ascending aorta—including the aortic root—by coronary re-implantation, and a segmental replacement of the aorta above the STJ, depends on the diameters at different sites of the aorta, in particular the SOV. In cases of total replacement, the choice between a valve-sparing intervention and a composite graft with a valve prosthesis depends on the analysis of aortic valve function and anatomy, the size and site of TAA, life expectancy, desired anticoagulation status, and the experience of the surgical team [17].

1.7.2.2 Aortic root

The goal of aortic valve–sparing root replacement procedures is preservation of native aortic leaflets to avoid prosthetic valve–related complications, while replacing the entire diseased proximal aortic wall to treat aortic root pathology.

Aortic root replacement (ARR) indications differ, but the selection of the optimal surgical approach is more complicated. The goal of these procedures is patient survival and prevention of late complications. The aneurysmal dilatation of the aortic root in congenital disorders (annuloaortic ectasia and bicuspid aortic valve) or in chronic aortic dissection is the most common indication for the ARR. Sinus of Valsalva aneurysms can be complicated with an aortocardiac fistulae, and ARR is the essential option to correct this pathology. Complicated aortic valve endocarditis with periannular abscess and/or fistulisation into the adjacent cardiac chambers sometimes requires lifesaving ARR. Root replacement remains the only option for extremely calcified ascending aortas with existing aortic valve pathologies. Root replacement would also be the appropriate surgical solution for the patient with a narrow aortic root. Finally, patients with congenital cardiac anomalies may require ARR in their lifetime because of the progressive root and ascending aorta dilatation that often occurs before (conotruncal abnormalities) or after (Ross, arterial switch) surgical correction [199].

The Standard remodelling technique developed by Yacoub conserves the native AV and re-creates aortic sinuses during total ARR without annular stabilisation. The Reimplantation technique developed by David also conserves the native AV but stabilises the aortic annulus and re-creates neo-pseudosinuses. To simplify AV–sparing operations, several techniques have been described, such as Florida sleeve repair, Corset technique, and (personalized external aortic root support) PEARS application [199].

Aortic root replacement is associated with high mortality and morbidity and is therefore frequently avoided in cases of acute aortic dissection for fear of increased surgical risk [200]. Approximation of

the aortic wall layers within the dissected SOV with a biological glue and subsequent supracoronary aortic replacement offers a simple and efficient method of preserving the native valve and abolishing the aortic insufficiency when it is caused by the distortion of root anatomy. However, non-curative root repair can result in late development of several pathologies, which, especially after use of glue, necessitating challenging redo surgeries.

The initial decision regarding the management of the aortic root in type A aortic dissection (TAAD) is whether to repair or replace the dissected sinus segments [201]. The standard indications for ARR in the setting TAAD are extensive tissue destruction, the presence of a concomitant aortic root aneurysm ≥45mm, or a known connective tissue disorder. The most common pathology observed is a primary intimal tear located in the ascending aorta with extension of the dissection flap into the noncoronary cusp, and relative preservation of the left and right coronary sinuses. Rarely are the aortic valve cusps or annulus impacted by the dissection process [201]. More recently, aortic valve–sparing operations have become a viable alternative in the setting of aortic root dilatation associated with pure aortic regurgitation. These techniques, however, are limited to patients with pliable valve cusps, thereby precluding many patients with BAV disease [202].

Cosgrove and colleagues [189] used techniques of valve repair in patients with a bicuspid, regurgitant aortic valve and published excellent early results. Others, using similar techniques, reported an incidence of reoperation approaching 50% in the early and intermediate postoperative phase [9]. The researchers found recurrent valve regurgitation particularly in conjunction with dilatation of the aortic root and attributed the repair failures to this aortic pathology.

Despite the BAV anatomy being considered less suitable by many surgeons for repair because of its limited functional prognosis, several authors have examined the feasibility of repairing leaking bicuspid aortic valves, irrespective of the presence or absence of concomitant aortic root or ascending aorta dilatation [189]. It is now more commonly reported that patients with a dilated aortic root and aortic regurgitation because of a bicuspid valve, with the combined application of valve reconstruction and root remodelling leads to good early results [189, 190].

Summaries of recommendations for the repair of TAA and aortic root aneurysms are show in the in the table below [Table 1.7].

Recommendations	Class	Level
Surgery is indicated in patients who have aortic root aneurysm, with maximal aortic diameter > or equal to 50mm for patients with MFS	I	C
Surgery should be considered in patients who have aortic root aneurysm, with maximal ascending aorta diameters: ➢ > or equal to 45mm for patients with MFS with risk factors ➢ > or equal to 50mm for patients with BAV with risk factors ➢ > or equal to 55mm for other patients with no elastopathy	IIa	C
Lower thresholds for intervention may be considered according to body surface area in patients of small stature or in the case of rapid progression, AR, planned pregnancy, and patients' preference	IIb	C

Table 1.7: Recommendations on intervention on ascending aortic aneurysms from the ESC guidelines [17].

1.7.2.3 Aortic stenosis

The choice of the intervention for AS should consider the cardiac and extracardiac characteristics of the patient, the individual risk of surgery, the feasibility of TAVR and the local experience and outcome data.

Available data from randomised controlled trials (RCT) and large registries in elderly patients at increased surgical risk show that TAVR is superior in terms of mortality to medical therapy in extreme-risk patients, non-inferior or superior to surgery in high-risk patients and non-inferior to surgery and even superior when transfemoral access is possible in intermediate and low-risk patients. In the two large studies on intermediate risk, the mean ages of patients were 82 and 80 years [145, 203] mean STS scores were 5.8% and 4.5%, and a high percentage were considered frail. Thus, the results are valid only for comparable patient groups.

Overall, rates of vascular complications, pacemaker implantation and paravalvular regurgitation were significantly higher for TAVR [204]. On the other hand, severe bleeding, acute kidney injury and new-onset atrial fibrillation (AF) were significantly more frequent with surgery, whereas no difference was observed in the rate of cerebrovascular events. In the latest Partner 3 trial results at 2 years, TAVR maintained superiority for the primary endpoint but not for the individual components, except for rehospitalisation. Between years 1 and 2, four TAVR patients died of cardiovascular causes (sudden cardiac death, fatal intracranial bleed secondary to fall, cardiac arrest secondary to hip surgery, and unknown) and three of non-cardiovascular causes (cancer, suicide,

and sepsis). In the surgical arm, one patient died of heart failure and two of unknown cardiovascular causes; none died of non-cardiovascular causes [146].

The favourable results of TAVR have been reproduced in multiple large-scale, nationwide registries supporting the generalisability of outcomes observed in RCTs. This favours the use of TAVR over surgery in elderly patients at increased surgical risk. However, the final decision between SAVR and TAVR (including the choice of access route) should be made by the Heart Team after careful individual evaluation [195].

The surgical management of AS with an AVR has been the evidence-based gold standard since 1961 when the first successful AVR was performed. Prior attempts at preservation of the native AV in 1956 via commissurotomy, decortication and refurbishment of stenosed valves were unsuccessful with rapid incompetency, recalcification and restenosis; and later attempts with the ball-valve prosthesis by Harken and colleagues in 1960 and Starr and Edwards in 1961 resulted in high operative mortality [205].

In the first series of AVR performed at Cleveland Clinic a total of 117 AVR's were performed with the primary indication being dominant AR and acquired AS. Age ranged from 10-75 years with 79/117 (68%) occurring in patients aged 41-60 years, and <1% being performed in patients over 70 years [205]. Morbidity and mortality were high in this series. Of the 117 AVR patients, 12% died in hospital, and 27% died in hospital or within 6 months. Interestingly mortality was lower (10% and 18% respectively) in a mitral valve replacement group of 97 patients.

Up until 2002, SAVR was performed routinely in all patients with severe AV disease, in addition to balloon aortic valvuloplasty (BAV) which was performed in patients with inoperable AS, but restenosis occurring in most cases within a year. Since 2002, TAVR has become an evolving option in the management of AS in what began as a high surgical risk alternative to a current day alternative to SAVR.

The latest AHA/ASC Valvular heart disease guidelines provide clear recommendations as to the indications for AVR in patients with AS, including the timing of intervention for AS in both SAVR and TAVR. Periodic monitoring is indicated in all patients in whom AVR is not yet indicated, including those with asymptomatic (Stage C) and symptomatic (Stage D) AS and those with low gradient AS (Stage D2 or D3) who do not meet the criteria for intervention (Figure 1.42) [198].

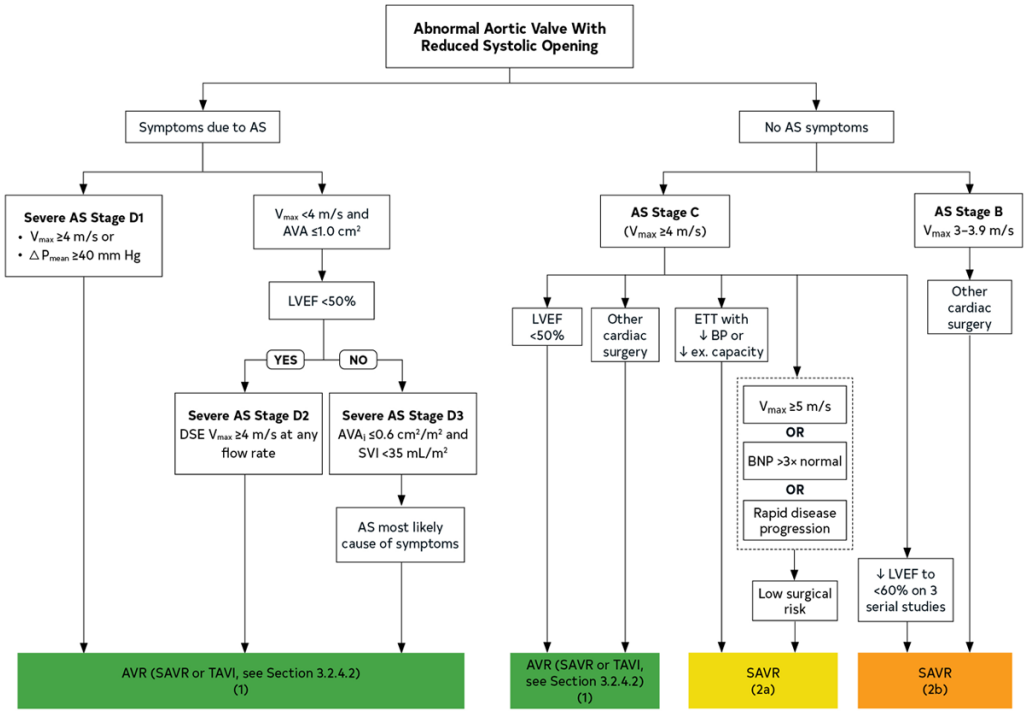

Figure 1.42: Flow chart demonstrating the management of severe AS per the 2020 ACC/AHA guidelines on the management of valvular heart disease [198] *AS indicates aortic stenosis; AVA, aortic valve area; AVAi, aortic valve area index; AVR, aortic valve replacement; BNP, B-type natriuretic peptide; BP, blood pressure; DSE, dobutamine stress echocardiography ETT, exercise treadmill test; LVEF, left ventricular ejection fraction; ⊥Pmean, mean systolic pressure gradient between LV and aorta; SAVR, surgical aortic valve replacement; SVI, stroke volume index; TAVI, transcatheter aortic valve implantation; TAVR.*

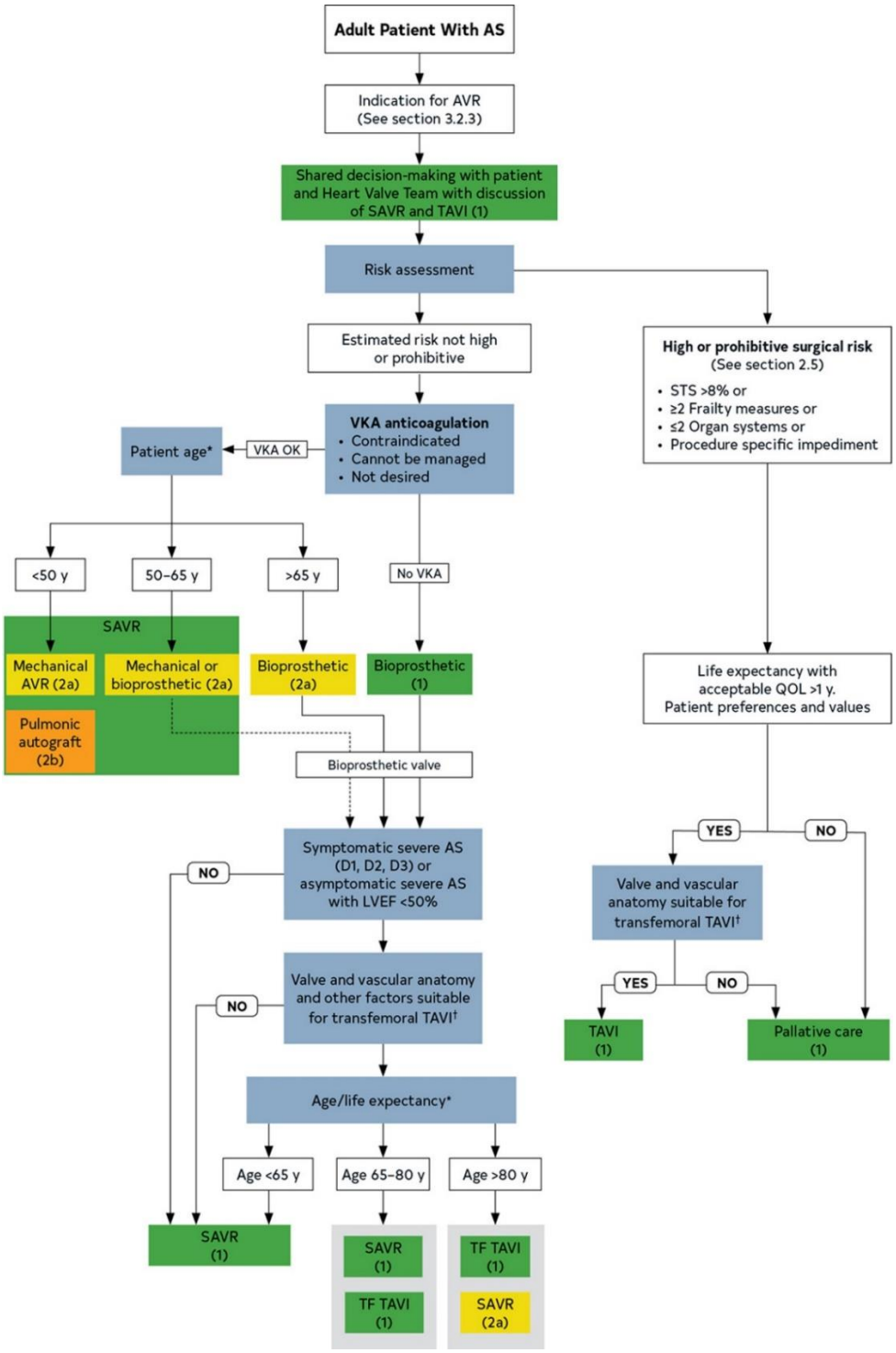

Figure 1.43: Choice of SAVR versus TAVR when AVR is indicated for valvular AS per the 2020 ACC/AHA guidelines on the management of valvular heart disease [198]. *AS indicates aortic stenosis; AVR, aortic valve replacement; LVEF, left ventricular ejection fraction; QOL, quality of life; SAVR, surgical aortic valve replacement; STS, Society of Thoracic Surgeons; TAVI, transcatheter aortic valve implantation; TF, transfemoral; and VKA, vitamin K antagonist.*

1.7.2.4 Surgical versus transcatheter replacement of the aortic valve

The most well-known clinical trials reporting on clinical outcomes in TAVR are the PARTNER trials. The non-inferiority designed PARTNER 1 trial in 2015 reported on outcomes in SAVR and TAVR in high-risk patient groups (mean STS score of 11.5%). The primary outcome of the trial was all cause mortality at 1 year, with secondary endpoints being stroke, readmission, acute kidney injury (AKI), vascular complications, and bleeding events. Periprocedural stroke or transient ischemic injury (TIA) was higher in TAVR (5.5%) versus SAVR (2.4%), and transapical TAVR had higher mortality compared to transapical SAVR. However, at 5 years there was no significant difference in all cause or cardiovascular mortality, stroke, or re-admission between SAVR and TAVR. Moderate or severe AR caused by paravalvular regurgitation was more common in the TAVR group and was associated with lower survival [144]. Author and investigator reasoning for the differences between paravalvular leak and clinical outcomes relate to valve development, operator expertise and experience, and patient selection for such trials.

Study	
Partner 1 [144]	➢ **High risk SAVR and TAVR with mean STS score 11.5%**
Inclusion criteria	• Senile degenerative AV stenosis with echocardiography derived criteria: mean gradient >40 mm Hg or jet velocity > 4.0 m/s or an aortic valve area (AVA) of < 0.8 cm2 (or AVA index < 0.5 cm2/m2) • Symptomatic due to AV stenosis as demonstrated by NYHA Functional Class ≥ II
Exclusion criteria	• Evidence of an acute myocardial infarction (MI) ≤ 1 month before the intended treatment • Aortic valve was a congenital unicuspid or congenital BAV, or was non-calcified. • Mixed AV disease • Any therapeutic invasive cardiac procedure performed within 30 days of the index procedure • Pre-existing prosthetic heart valve in any position, prosthetic ring, severe mitral annular calcification, or severe (greater than 3+) mitral regurgitation • Blood dyscrasias or history of bleeding diathesis or coagulopathy • Untreated clinically significant coronary artery disease requiring revascularisation • Haemodynamic instability • Need for emergency surgery for any reason • Hypertrophic cardiomyopathy with or without obstruction • Severe ventricular dysfunction with LVEF < 20% • Echocardiographic evidence of intracardiac mass, thrombus, or vegetation • Active peptic ulcer or upper gastro-intestinal bleeding within the prior 3 months • A known hypersensitivity or contraindication to aspirin, heparin, ticlopidine (Ticlid), or clopidogrel (Plavix), or sensitivity to contrast media, which cannot be adequately pre-medicated • Native aortic annulus size < 18mm or > 25mm as measured by echocardiogram • Recent (within 6 months) cerebrovascular accident or transient ischemic attack • Renal insufficiency (creatinine > 3.0mg/dL) and/or end stage renal disease requiring chronic dialysis • Life expectancy < 12 months due to non-cardiac co-morbid conditions • Significant abdominal or thoracic aorta disease, including aneurysm (defined as maximal luminal diameter 5cm or greater), marked tortuosity (hyperacute bend), aortic arch atheroma (especially if thick [> 5 mm], protruding or ulcerated), narrowing of the abdominal aorta (especially with calcification and surface irregularities), or severe "unfolding" and tortuosity of the thoracic aorta

	• Iliofemoral vessel characteristics that would preclude safe placement of 22F or 24F introducer sheath such as severe calcification, severe tortuosity, or vessels size diameter < 7 mm for 22F sheath or < 8mm for 24F sheath • Currently participating in an investigational drug or another device study • Active bacterial endocarditis or other active infections • Bulky calcified AV leaflets in close proximity to coronary ostia
Outcomes	• At 1 year, the rate of death from any cause was 30.7% with TAVR, as compared with 50.7% with standard therapy (p<0.001) • The rate of the composite end point of death from any cause or repeat hospitalisation was 42.5% with TAVR as compared with 71.6% with standard therapy (p<0.001) • Among survivors at 1 year, the rate of cardiac symptoms (New York Heart Association class III or IV) was lower among patients who had undergone TAVR than among those who had received standard therapy (p<0.001) • At 30 days, TAVR, as compared with standard therapy, was associated with a higher incidence of major strokes (p<0.001) • In the year after TAVR, there was no deterioration in the functioning of the bioprosthetic valve, as assessed by evidence of stenosis or regurgitation on an echocardiogram
Partner 2 [145]	➢ **Intermediate risk SAVR and TAVR with mean STS score 5.8%**
Inclusion criteria	• Patient had senile degenerative AV stenosis with echocardiographically derived criteria (mean gradient > 40 mmHg or jet velocity greater than 4.0 m/s and an initial AV area (AVA) of < 0.8 cm2 or indexed EOA < 0.5 cm2/m2) • Qualifying echo was within 60 days of the date of the procedure • Patient was symptomatic from his/her AV stenosis, as demonstrated by NYHA Functional Class II or greater • The heart team agreed (and verified in the case review process) that valve implantation would likely benefit the patient • Patient agreed to follow up and informed consent provided
Exclusion criteria	• Heart Team assessment of inoperability (including examining cardiac surgeon) • Evidence of an acute MI ≤ 1 month (30 days) before the intended • treatment • Aortic valve is a congenital unicuspid or congenital BAV, or is non-calcified • Mixed AV disease (AS and AR with predominant aortic regurgitation >3+) • Pre-existing mechanical or bioprosthetic valve in any position • Complex coronary artery disease (Unprotected left main coronary artery, syntax score > 32 in the absence of prior revascularization) • Any therapeutic invasive cardiac procedure performed within 30 days of the index procedure • Any patient with a BAV within 30 days of the procedure • Patients with planned concomitant surgical or transcatheter ablation for AF • Leukopenia (WBC < 3000 cell/mL), acute anaemia (Hgb < 9 g/dL), thrombocytopenia (Plt < 50,000 cell/mL) • Hypertrophic cardiomyopathy (HOCM) with or without obstruction • Severe ventricular dysfunction with LVEF < 20% • Echocardiographic evidence of intracardiac mass, thrombus, or vegetation • Active upper GI bleeding within 3 months (90 days) prior to procedure • A known contraindication or hypersensitivity to all anticoagulation regimens, or inability to be anticoagulated for the study procedure • Native aortic annulus size < 18 mm or > 27 mm as measured by echocardiogram • Clinically (by neurologist) or neuroimaging confirmed stroke or transient ischemic attack (TIA) within 6 months (180 days) of the procedure • Renal insufficiency (creatinine > 3.0 mg/dL) and/or renal replacement therapy at the time of screening • Estimated life expectancy < 24 months (730 days) due to carcinomas, chronic liver disease, chronic renal disease, or chronic end stage pulmonary disease • Expectation that patient will not improve despite treatment of AS • Active bacterial endocarditis within 6 months (180 days) of procedure • Patient refuses AVR surgery
Outcomes	• There was no significant difference in the primary end points of death and disabling stroke between SAVR and TAVR (p=0.25) • At 30 days, vascular complications were more frequent in TAVR (7.9%) versus SAVR (5%) (p=0.008)

	• Life-threatening bleeding was reported to have occurred more frequent in SAVR (43%) versus TAVR (10%) (p<0.001) as well as new onset AF in SAVR (26%) versus TAVR (9%) (p<0.001) • The need for PPM was higher in TAVR (8.5%) than in SAVR (6.9%) (p=0.17) • The frequency and severity of paravalvular AR was greater after TAVR (22.5% mild and 3.7% severe) versus SAVR (p<0.001) • The severity of paravalvular leak worsened at 2 years in the TAVR group, and those who had moderate to severe regurgitation had higher mortality within 2 years. (p<0.001). When explored in more detail, mild paravalvular leak worsened from 30 days to 2 years in the TAVR group in a reduced number of patients with supporting echocardiographic findings • Moderate or severe paravalvular leak worsened from 30 days to 2 years in the TAVR group, while in the SAVR group; mild, moderate, or severe paravalvular leak improved over time (p<0.001)
Partner 3 [146]	➢ **Low risk SAVR and TAVR with mean STS 1.9%**
Inclusion criteria	• Severe, calcific AS meeting the following criteria (AVA ≤ 1.0 cm2 or AVA index ≤ 0.6 cm2/m2, Jet velocity ≥ 4.0 m/s or mean gradient ≥ 40 mmHg, and • NYHA Functional Class ≥ 2, or • Exercise tolerance test that demonstrates a limited exercise capacity, abnormal blood pressure response, or arrhythmia, or • Asymptomatic with LVEF <50% • Heart team agrees the patient has a low risk of operative mortality and an STS < 4
Exclusion criteria	• Native aortic annulus size unsuitable for sizes 20, 23, 26, or 29mm transcatheter heart valve (THV) • Iliofemoral vessel characteristics that would preclude safe passage of the introducer sheath • Evidence of an acute MI ≤ 1 month (30 days) before randomisation • AV is unicuspid, bicuspid, or non-calcified • Severe AR (>3+) • Severe mitral regurgitation (>3+) or ≥ moderate stenosis • Pre-existing mechanical or bioprosthetic valve in any position • Complex coronary artery disease (Unprotected left main coronary artery, syntax score > 32 in the absence of prior revascularization) • Heart Team assessment that optimal revascularisation cannot be performed • Symptomatic carotid or vertebral artery disease or successful treatment of carotid stenosis within 30 days of randomisation • Leukopenia (WBC < 3000 cell/mL), anaemia (Hgb < 9 g/dL), thrombocytopenia (Plt < 50,000 cell/mL), history of bleeding diathesis or coagulopathy, or hypercoagulable states • Haemodynamic or respiratory instability within 30 days of randomisation • Hypertrophic cardiomyopathy (HOCM) with obstruction • Ventricular dysfunction with LVEF < 30% • Cardiac imaging (echo, CT, and/or MRI) evidence of intracardiac mass, thrombus, or vegetation • Inability to tolerate, or condition precluding treatment with, antithrombotic or anticoagulation therapy during or after the valve implant procedure • Stroke or transient ischemic attack (TIA) within 90 days of randomisation • Renal insufficiency (eGFR < 30 ml/min per the Cockcroft-Gault formula) and/or renal replacement therapy at the time of screening • Active bacterial endocarditis within 180 days of randomisation • Severe lung disease (FEV1 < 50% predicted) or currently on home oxygen • Severe pulmonary hypertension (PA systolic pressure ≥ 2/3 systemic pressure) • History of cirrhosis or any active liver disease • Significant frailty as determined by the Heart Team (after objective assessment of frailty parameters) • Significant abdominal or thoracic aortic disease (such as porcelain aorta, aneurysm, severe calcification, aortic coarctation) that would preclude safe passage of the delivery system or cannulation and aortotomy for surgical AVR • Hostile chest or conditions or complications from prior surgery that would preclude safe reoperation (mediastinitis, radiation damage, abnormal chest wall, adhesion of aorta or IMA to sternum) • Patient refuses blood products • BMI > 50 kg/m2 • Estimated life expectancy < 24 months • Absolute contraindications or allergy to iodinated contrast that cannot be adequately treated with pre-medication

	• Immobility that would prevent completion of study procedures (e.g., six-minute walk test)
Outcomes	• At 1 year, death from any cause was higher in the SAVR group (2.5%) versus the TAVR group (1%). Stroke was higher in the SAVR group (3.1%) versus the TAVR group (1.2%) (p<0.001) • Rehospitalisation was higher in the SAVR group (11%) versus TAVR group (7.3%) (p=0.001) • The percentage of patients with new left bundle-branch block at 1 year was 23.7% in the TAVR group as compared with 8.0% in the surgery group (hazard ratio, 3.43; 95% CI, 2.32 to 5.08) • The percentage of mild paravalvular regurgitation at 1 year was higher in the TAVR group (29.4%) versus the SAVR group (2.1%). (p-value/CI not provided)
	•
Observant study [206]	➢ **SAVR versus TAVR in low-risk patients 3-year outcomes**
Inclusion criteria	• Diagnosis of severe AV stenosis (defined as an aortic valve area <1 cm2, maximum aortic velocity >4 m/s, or mean pressure gradient >40 mm Hg) and requiring an AVR • Patients with Euro SCORE II <4%
Exclusion criteria	• Porcelain aorta • Hostile chest • Active endocarditis • Oxygen therapy • Undergoing any combined procedure (coronary revascularisation or intervention on other heart valves) • Patients who underwent emergency procedure
Outcomes	• Thirty-day mortality was 2.9% after SAVR and 2.6% after TAVI (P=0.82) • One-, 2-, and 3-year survival were 92.2%, 87.2%, and 83.4% after SAVR and 88.6%, 80.4%, and 72.0% after TAVR, respectively (stratified log-rank test; P<0.001) • Propensity score–adjusted analysis performed on the overall low-risk population showed that TAVR was associated with significantly lower 3-year survival than SAVR (P=0.002, hazard ratio =1.59, 95% confidence interval: 1.18–2.13) • Stroke rate was rather low and similar in the 2 study groups (SAVR 1.1% versus TAVR 1.1%; P=1.00) • The rates of cardiac tamponade (4.3% versus 1.7%; P=0.049), PPM implantation (12.7% versus 2.6%; P<0.001), major vascular damage (7.6% versus 0%; P<0.001), mild to- severe paravalvular regurgitation (48.2% versus 11.3%; P<0.001), and moderate-to-severe paravalvular regurgitation (9.7% versus 1.5%; P<0.001) were significantly higher after TAVR compared with SAVR • TAVR was associated with significantly lower risk of cardiogenic shock (1.7% versus 4.6%; P=0.025), severe bleeding (4.4% versus 15.2%; P<0.001), and acute kidney injury (AKIN stages 1–3: 26.0% versus 43.7%; P<0.001) compared with SAVR • TAVR was associated with lower mean transvalvular gradient (10.6 versus 14.4 mm Hg; P<0.001)

Table 1.8: Randomised controlled trial (RCT) Partner trials 1-3 and real-world study (OBSERVANT study) comparing SAVR and TAVR outcomes in high intermediate and low risk groups.

The Partner 2 trial in 2016 reported on outcomes in SAVR and TAVR in intermediate-risk groups (mean STS score of 5.8). Similarly, the primary outcome of the trial was death of any cause or disabling stroke at 2 years, with secondary endpoints being vascular complications, life-threatening bleeding, AKI, new onset AF, re-admissions, PPM implantation, length of stay and paravalvular AR in addition to others. There was no significant difference in the primary end points of death and disabling stroke between SAVR and TAVR. At 30 days, vascular complications were more frequent in TAVR (7.9%) versus SAVR (5%). Life-threatening bleeding was reported to have occurred more

frequent in SAVR (43%) versus TAVR (10%), as well as new onset AF in SAVR (26%) and in TAVR (9%). The need for PPM was higher in TAVR (8.5%) than in SAVR (6.9%). The frequency and severity of paravalvular AR was greater after TAVR (22.5% mild and 3.7% severe) versus SAVR. The severity of paravalvular leak worsened at 2 years in the TAVR group, and those who had moderate to severe regurgitation had higher mortality within 2 years [145]. Trial conclusions was that SAVR and TAVR outcomes in respect to death and disabling stroke are similar in intermediate-risk patients, and it was deemed that the TAVR expandable prosthesis may reduce patient prosthetic mismatch and result in greater long-term outcomes, and the paravalvular leak only resulted in increased mortality in the moderate to severe TAVR group which was less than 4%.

The Partner 3 trial in 2019 reported on outcomes in SAVR and TAVR in low-risk groups (mean STS score 1.9%). As in the previous trials, the primary outcome was death, stroke, or rehospitalisation at 1 year; with secondary endpoints being new onset AF at 30 days, length of hospital stay, improvement in heart failure symptoms and functional outcomes as measured by a 6-minute walk test. At 1 year, death from any cause was higher in the SAVR group (2.5%) versus the TAVR group (1%). Stroke was higher in the SAVR group (3.1%) versus the TAVR group (1.2%). Rehospitalisation was higher in the SAVR group (11%) versus TAVR group (7.3%). Trial conclusions was that among patients with severe AS who were at low risk for death with surgery, the rate of composite death, stroke, or rehospitalisation at 1 year was significantly lower with TAVR than with SAVR [146]. Exclusion criteria in the Partner trials are extensive especially when considering current day patient cohorts and associated presentations and comorbidities (Table 1.8).

Several other real-world studies have reviewed the SAVR and TAVR outcomes in all risk groups. The OBSERVANT study in 2016 reported outcomes in patients of low surgical risks (Table 1.8). Improved 3-year survival was better in SAVR (83.4%) versus TAVR (72%), and freedom from major cardiac and cerebrovascular events was greater in SAVR (80.9%) versus TAVR (67.3%) [206]. The Notion trial in 2015 [207] reported outcomes in patients of high surgical risk. They found no significant difference between SAVR and TAVR in the areas of composite death rate of any cause, stroke, or MI after 1 year. The SURTAVI trial in 2017 [203] reported outcomes in patients of intermediate surgical risk. The incidence of the primary end point at 2 years was 12.6% in the TAVR group and 14% in the SAVR group; with TAVR deemed a suitable non-inferior alternative to SAVR in this patient group.

Several institutions have reported on their own registries (state based and national) comparing SAVR and TAVR outcomes in the evolving TAVR field. The Italian Observant study reported 5-year outcomes [208]. At 5 years, the rate of death from any cause was 35.8% in SAVR and 48.3% in TAVR ($p=0.002$). In addition, TAVR was associated with increased risk of major adverse cardiac and cerebrovascular events (54%) versus SAVR (42.5%). The transcatheter valve registry and STS national database in the US compared SAVR and TAVR in intermediate and high-risk cohorts [209]. In both SAVR and TAVR, there was no significant difference in rates of death (17.9% versus

17.3%), and stroke (3.3% versus 4.2%). The Nationwide Finnish Registry of Transcatheter and Surgical Aortic Valve Replacement for Aortic Stenosis (FinnValve Registry) reported outcomes in SAVR and TAVR patients of low surgical risk [210]. Mortality at 30-days was 3.6% in SAVR and 1.3% in TAVR. Three-year survival was 87.7% in SAVR and 85.7% in TAVR.

Thourani and colleagues [211] published an update on the European registries in outcomes of AVR via SAVR and TAVR approaches. The German Aortic Valve Registry (GARY) was established in 2010. One-year follow up demonstrated excellent results in the SAVR group outcomes, and TAVR was deemed a good alternative for elderly and high-risk patients. Severe complications in TAVR patients have steadily decreased over time [212]. The French Registry, in an early analysis in 2009 on high-risk patients with a high predictive operative mortality (18.9%) and mean age of 82 years reported a 12.7% 30-day operative mortality and initial stroke rate of 3.7%. In 2010, in the FRANCE2 trial, 30-day operative mortality reduced to 9.7%- and 1-year mortality was 24% in a similar high risk, elderly cohort. The major stroke rate had decreased to 2.3%. The United Kingdom (UK) registry was initiated in 2007. By 2009 they reported a 96% 30-day survival in patients undergoing TAVR, 1-year survival was 78% and 2-year survival 73%, 3-year survival 61% and 5-year survival 45%, which was deemed respectable. When comparing SAVR and TAVR, Grant and colleagues in 2016 observed a 30-day mortality of 2.1% in SAVR and 6.2% in TAVR as well as 5-year survival rates of 82% and 46% respectively [213]. The Canadian Registry evidenced relatively high mortality rates associated with TAVR in extreme-risk patients at mid-to long term follow up (24% at 1 year, 56% at 4 years). The main predictors in poorer late outcomes were chronic obstructive pulmonary disease (COPD), chronic kidney disease (CKD), chronic AF and frailty as reported by an "eyeball test". This highlighted the importance of patient selection, however in the FRANCE2 trial or PARTNER trial no objective measures of frailty were included in TAVR risk scores despite frailty being recognised as an important prognostic factor in TAVR patients [211].

The most recent review of the Australian and New Zealand Society of Cardiothoracic Surgery (ANZSCTS) database was data obtained from 2009-2015 and published in 2016, which extrapolated data from 733 patients at St Vincent's hospital (669 SAVR patients and 64 TAVR patients). Primary end points were 30-day mortality and 2-year survival with secondary endpoints looking at readmission within 30-days, new AF, heart block requiring PPM, significant paravalvular leak (> mild AR), stroke, pneumonia, and blood transfusion requirements [214]. Survival at 2 years was 74% for TAVR and 80% in SAVR (in propensity matched pairs which yielded 44 pairs). In the propensity matched analysis, 30-day mortality was 5% in both groups, requirement of PPM was higher in TAVR at 23% and 5% in SAVR, postoperative AF was higher in SAVR at 41% and 2% in TAVR. The rates of paravalvular leak were 7% in TAVR and 0% in SAVR. Lack of statistical significance in the leak rate is likely due to lack of statistical power. In this analysis, TAVR patients included were of high operative risk and no validated frailty score was used to guide treatment allocation.

The latest guidelines are shown in Figure 1.43.

1.8 Rationale of Present work

Aneurysms of the aortic root and ascending aorta are treated as a single entity and managed under the same guidelines despite differences in embryology, structure, function, and often the complexity of surgeries performed.

This is likely because the aortic root and ascending aorta are a continuous structure, and the approach to surgical management can be similar in certain circumstances. Therefore, there is a need to determine if the differences between the aortic root and ascending aorta exist:

(1) In the macroscopic integrity of the aortic wall as determined by animal and human pressure testing and MRI measurement of wall sheer stress (WSS), and

(2) In the microscopic structure of collagen and elastin content and types determined by histology and immunohistochemistry.

Aortic valve disease in current practice is treated by surgical or transcatheter methods, with evidence indicating an increasing role for transcatheter techniques. There is a need to explore the complications and outcomes that influence recovery following aortic valve replacement through:

(1) Comparing the surgical complications between SAVR and TAVR in the ANZSCTS database, and

(2) Determining the frailty and patient related outcome measures between SAVR and TAVR over a 12-month period.

CHAPTER TWO

Histological regional analysis of the aortic root and thoracic ascending aorta: a complete analysis of aneurysms from root to arch

Published in The Journal of Cardiothoracic Surgery, September 2021

2.1 Statement of authorship

Statement of Authorship

Title of Paper	Can the histology of the aortic root and ascending aortic aneurysms guide us in clinical management? A complete histological and immunohistochemistry analysis from root to arch
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Publication Details	Original publication submitted to the Journal of Pathology

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Name of Principal Author (Candidate)	Timothy Luke Surman		
Contribution to the Paper	Primary investigator and author Specimen preparation Histological analysis Publication formulation		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	13/2/21

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Publication planning Publication editing		
Signature		Date	17/2/21

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Contribution to the Paper	Primary investigator and author Specimen preparation Histological analysis Publication formulation		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
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Please cut and paste additional co-author panels here as required.

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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 13/2/21

Co-Author Contributions

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i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Name of Co-Author	James Edwards	
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2.2 Manuscript summary

Title

Histological regional analysis of the aortic root and thoracic ascending aorta: A complete analysis of aneurysms from root to arch

Authors name and affiliations

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2.3 Abstract

Background

Although aortic root and ascending aortic aneurysms are treated the same, they differ in embryological development and pathological processes.

This study examines the microscopic structural differences between aortic root and ascending aortic aneurysms, correlating these features to the macroscopic pathophysiological processes.

Methods

We obtained surgical samples from ascending aortic aneurysms (n = 11), aortic root aneurysms (n = 3), and non-aneurysmal patients (n = 7), Aortic collagen and elastin content were examined via histological analysis, and immunohistochemistry techniques used to determine collagen I, III, and IV subtypes. Analysis was via observational features, and colour deconvolution quantification techniques.

Results

Elastin fibre disruption and fragmentation was the most extensive in the proximal aneurysmal regions. Medial fibrosis and collagen density increased in proximal aneurysmal regions and aortic root aneurysms ($p < 0.005$). Collagen I was seen in highest quantity in aortic root aneurysms. Collagen I content was greatest in the sinus tissue regions compared to the valvular and ostial regions ($p < 0.005$) Collagen III and IV quantification did not vary greatly. The most susceptible regions to ultrastructural changes in disease are the proximal ascending aorta and aortic root.

Conclusions

The aortic root differs histologically from the ascending aorta confirming its unique composition in aneurysm pathology. These findings should prompt further evaluation on the influence of this altered structure on function which could potentially guide clinical management.

Keywords

Aortic root, Ascending aorta, Aneurysms, Histology, Immunohistochemistry

2.4 Background

Dissection of either the ascending aorta or aortic root can have catastrophic consequences, and is associated with a high mortality. The aortic root and annulus are less commonly involved in the dissection process compared to the ascending aorta [1]. Aortic aneurysms involving either the ascending aorta or aortic root, predispose patients to aortic dissection [2], but the aortic root aneurysms are especially challenging, given their anatomical location. Consequently, aortic root aneurysms are associated with higher morbidity and mortality compared to those in the ascending aorta [3]. This difference in outcomes may be attributable to regional structural differences (embryological and histological) within the aortic wall, as well as differences in wall stress. Concerning the latter, ascending aorta pathology is most reported in the right lateral wall where the greatest shear force on the aortic wall occurs [4], whereas aortic root pathology is often an extension of the dissection flap into the noncoronary cusp [5]. Despite these structural and functional differences between ascending aortic and aortic root aneurysms, there is no differentiation in management plans in current clinical practice [6,7]. Thus, a greater understanding of aortic wall structure may influence treatments strategies for these heterogenous pathologies.

2.4.1 Normal aortic wall structure

The key microstructural components of the aortic wall are collagen and elastin. With age, the ascending aorta becomes stiffer, with incremental increases in collagen content [8,9,10]. Similarly, collagen becomes a crucial element within the aortic root, with elastin and collagen fibres in the intermediate layer of the commissures in the annulus [11,12,13,14,15]. The aortic root sinus layers are likened to the ascending aorta itself, with smooth muscle cells, elastic fibres, collagen II and III and proteoglycans within the media, and collagen I makes up the adventitia and intima [16]. The sinotubular junction (STJ) is described as having a thicker wall [17]. The two principal types of collagens found in the aorta are types I and III, accounting for 80–90% of the collagen content [18].

2.4.2 Aortic aneurysm pathology

Historically, pathological analysis of the aortic wall has been primarily observational (i.e., pattern recognition) with limited quantification of the microstructural elements [19]. Reported ascending aorta aneurysm pathology has included cystic medionecrosis, aortitis, varying defects in elasticity, fibrosis, elastin and collagen fibre degradation and transmural defects that seemed to predispose to partial dissections and rupture [20,21,22,23,24,25]. Aortic root pathology includes cystic medionecrosis, medial fragmentation, elastic fibre and collagen fragmentation, and mucoid accumulation [26,27]. Direct comparison between regions has described the ascending aorta as having tighter, denser weaves of elastin, and more irregular thickness than in the aortic sinus tissue. Collagen has more of a regular distribution in the ascending aorta compared with the aortic sinuses, and is in greater in proportions on the luminal side in both groups [28]. Observational analysis has shown many similarities between the ascending aorta and root in disease, but notable differences in collagen and elastin structure. Research to date has confirmed that observational analysis has lacked precision and specificity to the core proteins affected. Specifically, histological, and cytological staining by conventional methods loses considerable information, and analysis via biochemical assays and flow cytometry is destructive and morphology is often lost [29]. In addition, digital image analysis, and colour deconvolution is described as faster, more objective, and less laborious than visual inspection [29]. Digital image analysis has also been supported in determining collagen subtypes in immunohistochemistry [30]. This technique allowed differentiation between collagen types, the assessment of collagen orientation, and was deemed an easily reproducible technique [30]. Regional analysis of histopathology of the ascending aorta and aortic root has not been performed in detail, and no direct comparison have been made [31,32,33,34], but there have been reports that collagen types in the aortic root aneurysms change significantly; with collagen I and III decreasing and collagens XI and V increasing [26].

Considering the previously observed structural differences, this project aims to clarify qualitative and quantitative differences between aortic root and ascending aorta aneurysms in relation to (1) collagen and elastin composition, and (2) collagen subtypes.

2.5 Material and Methods

Ethics and governance approval was obtained from the Central Adelaide Local Health Care Network (CALHN) (HREC/18/CALHN/188), with research conducted at the Medical Device Research Institute, and University of Adelaide Histology department, Adelaide, South Australia. Data was collected from July 2019 to September 2020.

A total of 11 human aneurysmal samples were collected over this period (Table 2.1), 7 non-aneurysmal samples and 3 isolated aneurysmal aortic root specimens (Table 2.2) giving a total of 21 patients. Inclusion criterion was an isolated aortic surgical procedure as a non-emergency. Exclusion criteria included those undergoing a concomitant cardiac or thoracic procedure, or an emergency.

Age	**Sex**	**HTN**	**Diabetes**	**CVA**	**CKD**	**CAD**	**Aortic pathology**
69	Male	Yes	No	No	No	No	Tricuspid AV, dilated ascending aorta
78	Female	Yes	No	No	No	No	Tricuspid aortic valve, dilated ascending aorta
73	Male	Yes	No	Yes	No	No	BAV, dilated ascending aorta
53	Female	Yes	No	No	No	No	Tricuspid AV, dilated ascending aorta
83	Male	Yes	Yes	No	No	No	Tricuspid AV, dilated ascending aorta
75	Male	Yes	No	No	No	No	Tricuspid aortic valve, dilated ascending aorta
55	Male	Yes	No	No	No	No	Tricuspid AV, dilated ascending aorta
73	Female	Yes	Yes	No	No	No	Tricuspid AV, dilated ascending aorta
83	Female	Yes	Yes	No	No	No	Tricuspid AV, dilated ascending aorta
27	Male	No	No	No	No	No	BAV, dilated ascending aorta, MFS
45	Male	No	No	No	No	No	BAV, dilated ascending aorta
64.90	7M/4F						

Table 2.1: Preoperative demographics, medical comorbidities, and aortic pathology of 11 aortic aneurysm patients.

Age	Sex	HTN	Diabetes	CVA	CKD	CAD	Aortic pathology
46	Male	No	No	No	No	No	BAV, dilated aortic root
61	Male	Yes	No	No	No	No	Tricuspid aortic valve, dilated aortic root
69	Female	Yes	No	No	No	No	Tricuspid aortic valve, dilated aortic root
53.50	2M/1F						

Table 2.2: Preoperative demographics, medical comorbidities, and aortic pathology of 3 isolated aortic root patients.

2.5.1 Specimen preparation

Aneurysmal aortic tissue was obtained from operative specimens retrieved at the Cardiothoracic Surgical Unit at the Royal Adelaide Hospital, Adelaide, South Australia and non-aneurysmal aortic root and ascending aorta samples were obtained from cadaveric hearts provided by Science Care (Phoenix, Arizona, USA) as part of a tissue donation program. Specimen preparation occurred at the Medical Device Research Institute, Flinders University, and the University of Adelaide Medical School Histology Department. Aneurysmal ascending aortas were sectioned into proximal, middle, and distal regions. Aneurysmal root tissue was excised and separated into sinus and non-sinus (valvular/ostial) regions. Non-aneurysmal regions were divided into root, proximal ascending, mid ascending, and distal ascending aorta segments.

2.5.2 Histological and Immunohistological preparation

Tissue was placed in 10% neutral buffered formalin solution for fixation following preparation, embedded, and cut using a Leica rotary microtome (Leica Biosystems, Mt Waverley Australia) into 5 μm edge-to-edge sections. The basic histological stains and special stains used included Hematoxylin and Eosin (H&E), Van Gieson (EVG), Masson's Trichrome (Masson's), Alcian blue, and Von Kossa (VK) stains. Masson's' trichrome staining was completed with Celestin blue reagent, stained with biebrich scarlet-acid fuchsin and aniline blue solution, and differentiated in 1% acetic acid. Van Gieson (EVG) staining was oxidised with 0.5% potassium permanganate reagent, decolourised with oxalic acid, stained with miller's elastic stain, and counterstained with Curtis' stain.

For the immunohistochemical component, rabbit polyclonal antibodies to Collagen I (Abcam, Cambridge, UK. Cat # ab138492), Collagen III (Abcam, Cambridge, UK. Cat # ab7778) and Collagen IV (Abcam, Cambridge, UK. Cat # ab6586) were used. In brief, sections were dewaxed using xylene and then dehydrated through alcohols. Dehydrated sections were treated with Methanol/H2O2 for 30 min. The sections were then twice in phosphate buffered saline (PBS) (pH 7.4) for a further 5 min each wash. Antigen retrieval was then performed using Citrate Buffer (pH 6.0), and slides were allowed to cool before being washed twice in PBS (pH 7.4). All slides were then treated with Proteinase K (Merck Millipore, Cambridge, USA. Cat # 21627) for 15 min, then washed with PBS (pH 7.4). Following this process, all slides had non-specific proteins blocked using normal horse serum for 30 min. Collagen I antibody was applied at a dilution of 1/5000, Collagen III at 1/1000 and Collagen IV at 1/500. All antibodies were incubated overnight. The following day, all sections underwent two washes in PBS, then a biotinylated anti-rabbit secondary (Catalogue No. BA-1000, Vector Laboratories, USA) was applied to all sections. They were all incubated for 30 min at room temperature. Following the secondary incubation two PBS washes were carried out, all slides were incubated for a further 1 hour at room temperature with a streptavidin-peroxidase conjugate tertiary antibody (Cat No.127, Pierce, USA). Sections were washed under running tap water for 10 min. Sections were visualised using diaminobenzidinetetrahydrochloride (DAB), washed, counterstained with haematoxylin, dehydrated, cleared, and mounted on glass coverslips.

2.5.3 Qualitative analysis

Histological qualitative evaluation was undertaken by the primary investigator and a clinical histopathologist, with the following features particularly noted:

- intimomedial tear (dissecting aneurysm),
- insudation of plasma proteins/erythrocytes (PAS positive),
- elastic fibre disruption/fragmentation/diminution
- medial fibrosis,
- endothelium disruption/loss of integrity,
- thrombosis,
- subendothelial fibrosis,
- mineralisation (calcification),
- mural hyalinisation,
- mural fibrinoid necrosis,
- mucoid degeneration,

- chondroid metaplasia (cartilage disruption),
- neovascularization,
- cholesterol clefts,
- additional features.

Grading of individual structural components was determined using the classification system recommended by Catell and colleagues., with the degree of pathology denoted as mild, moderate, or severe, and the extension of this pathology denoted as focal, multifocal, or extensive [35].

2.5.4 Quantification analysis

Histological slides were scanned using Nanozoomer digital slide scanner (Hamamatsu Photonics), Zen Blue 3.0 (Zeiss) and NDP view 2.0 (Hamamatsu Photonics) depending on the slide size. Scanned histological slides were then analysed and quantified using Fiji by Image J (National Institutes of Health, USA). Quantification of elastin and collagen fibres then proceeded using the colour deconvolution plugin, whilst collagen type immunohistochemistry proceeded with the immunohistochemistry (IHC toolbox) plugin in Image J v.1.53 (The University of Nottingham, UK). The process involved in the quantification of collagen and elastin fibres included the following steps: image acquisition, scale setting, RGB colour space conversion, selection of the colour deconvolution toolbox, adjustment of the threshold value, measurement of the threshold area, quantification of the collagen or elastin fibres in the ROI, and imaging of the collagen and elastin fibre areas. Similarly, the process in quantification of collagen subtypes included image acquisition, scale setting, RGB colour space conversion, selection of the IHC toolbox, adjustment of the threshold value, measurement of the threshold area, quantification of the collagen subtypes in the ROI, and imaging of the collagen areas. Each measurement was performed twice to minimise quantification errors.

2.5.5 Statistical analysis

Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software, San Diego, California). A p-value of < 0.05 was considered significant. Non-parametric statistical test was utilised considering the skewed population sampled. Specific tests included the Wilcoxon test which was used to compare regional differences between proximal, middle, and distal ascending aorta aneurysms (Table 2.3), and the Mann–Whitney U test which was used to compare elastin and collagen content in the aortic root (Table 2.4), and collagen subtypes in the aortic root (Table 2.5).

Patient	Proximal (%)				Middle (%)				Distal (%)			
	Anterior	Posterior	Inner	Outer	Anterior	Posterior	Inner	Outer	Anterior	Posterior	Inner	Outer
1 -1	9.3	6.7	18.5	10.2	17.2	12.5	23.1	25.7	11.7	14.6	21.0	15.8
1-2	10.3	7.2	19.6	12.1	20.8	8.8	24.9	24.0	15.5	13.6	21.0	15.8
2-1	24.7	14.7	24.7	11.3	14.6	17.2	12.7	14.7	15.9	18.5	6.5	10.9
2-2	23.0	12.6	22.5	12.3	14.0	15.7	10.7	12.7	17.0	15.5	8.5	12.0
3-1	12.4	13.5	23.7	13.5	12.0	20.2	29.8	32.2	25.8	23.3	28.6	14.4
3-2	11.8	11.4	23.0	12.4	12.0	19.0	28.3	28.9	25.8	20.4	17.8	16.1
4-1	17.4	7.3	14.4	13.5	14.5	10.9	16.4	10.1	11.6	11.6	23.4	21.4
4-2	14.4	9.5	9.8	15.9	11.1	11.0	15.9	10.6	9.1	11.6	21.4	20.4
5-1	21.3	9.7	12.2	25.1	9.2	15.5	27.1	17.0	11.1	11.6	23.4	21.4
5-2	23.1	8.7	12.1	21.3	9.3	15.5	24.5	22.6	13.0	16.0	23.4	20.6
6-1	8.8	8.8	17.7	13.8	8.8	20.2	13.5	11.8	22.9	20.3	23.3	16.6
6-2	9.9	9.1	19.3	13.9	15.8	21.3	23.8	13.5	20.4	21.0	30.5	16.5
7-1	22.1	23.0	21.9	10.3	19.9	14.0	11.6	16.6	19.3	15.0	19.2	14.3
7-2	22.4	23.3	21.1	12.1	17.1	13.9	18.2	16.7	17.3	10.1	25.1	27.5
8-1	8.9	10.5	19.0	5.7	22.8	11.0	19.6	21.4	19.0	11.2	13.2	19.2
8-2	8.8	11.5	21.4	8.9	23.7	11.9	20.7	22.2	19.0	12.2	13.8	20.1
9-1	9.4	8.5	19.0	7.1	15.8	6.1	12.3	13.0	24.7	11.2	13.2	10.0
9-2	9.9	12.0	19.0	8.4	14.2	8.7	18.8	13.0	19.4	12.5	10.6	12.9
10-1	9.4	6.5	15.3	14.8	11.7	12.5	19.6	12.8	8.3	19.5	14.0	13.4
10-2	9.1	7.3	12.6	17.7	10.6	16.8	12.7	8.1	7.6	20.4	10.3	15.6
11-1	11.4	6.9	12.4	10.8	9.6	10.5	18.6	9.7	6.3	12.7	13.1	12.5
11-2	11.1	7.3	12.6	10.7	10.6	10.8	18.7	8.1	7.6	12.4	12.3	13.6
Average	**14.0**	**10.7**	**17.8**	**12.8**	**14.3**	**13.8**	**19.2**	**16.6**	**15.8**	**15.2**	**17.9**	**16.4**
Standard deviation	***5.85***	***4.64***	***4.42***	***4.39***	***4.42***	***4.11***	***5.64***	***6.86***	***6.13***	***4.03***	***6.70***	***4.23***

Table 2.3: Summary of colour deconvolution analysis in elastic tissue composition via EVG staining in aneurysmal patients.

Specimen	Location	EVG results (%)	Masson's trichrome results (%)
1-1	Aortic root sinus tissue	9.2	42.6
1-2	Aortic root sinus tissue	10.3	40.5
2-1	Aortic root sinus tissue	15.2	20.4
2-2	Aortic root sinus tissue	15.5	21.5
3-1	Aortic root sinus tissue	16.9	31.9
3-2	Aortic root sinus tissue	17.4	30.6
4-1	Aortic root sinus tissue	13.0	25.9
4-2	Aortic root sinus tissue	13.5	25.1
5-1	Aortic root sinus tissue (coronary ostia)	38.8	7.7
5-2	Aortic root sinus tissue (coronary ostia)	37.8	8.4
6-1	Aortic root sinus tissue	20.8	11.5
6-2	Aortic root sinus tissue	21.6	12.5
7-1	Aortic root sinus tissue (coronary ostia)	17.0	6.0
7-2	Aortic root sinus tissue (coronary ostia)	16.4	6.4
8-1	Aortic root sinus tissue	27.4	6.5
8-2	Aortic root sinus tissue	28.4	6.4
9-1	Aortic root sinus tissue	23.7	14.7
9-2	Aortic root sinus tissue	25.0	15.5
10-1	Aortic root sinus tissue	30.4	11.2
10-2	Aortic root sinus tissue	31.5	11.6
11-1	Aortic root sinus tissue	30.7	8.8
11-2	Aortic root sinus tissue	31.5	9.4
12-1	Aortic root sinus tissue	43.9	11.9
12-2	Aortic root sinus tissue	42.5	12.6
13-1	Aortic root sinus tissue (valvular tissue inferior)	6.1	7.5
14-1	Aortic root sinus tissue (valvular tissue inferior)	7.2	8.4
15-1	Aortic root sinus tissue (valvular tissue inferior)	8.2	13.4
16-1	Aortic root sinus tissue (valvular tissue inferior)	9.4	14.6
17-1	Aortic root sinus tissue	13.1	28.2
18-1	Aortic root sinus tissue	14.2	29.5
19-1	Aortic root sinus tissue	14.4	15.7
20-1	Aortic root sinus tissue	15.7	14.6
Average		**20.8**	**16.6**

Table 2.4: Summary of the colour deconvolution results from the aortic root aneurysm patients.

Specimen number	Tissue region	Collagen I (%)	Collagen III (%)	Collagen IV (%)
1-1	Aortic root sinus tissue	22.5	14.3	15.6
1-2	Aortic root sinus tissue	23.5	15.2	16.3
2-1	Aortic root sinus tissue (coronary ostium)	8.2	10.9	21.1
2-2	Aortic root sinus tissue (coronary ostium)	9.2	11.3	22.5
3-1	Aortic root sinus tissue (valve leaflets inferiorly)	10.5	16.7	17.7
3-2	Aortic root sinus tissue	11.5	18.5	19.5
4-1	Aortic root sinus tissue	15.7	8.8	9.9
4-2	Aortic root sinus tissue	16.5	9.5	10.5
5-1	Aortic root sinus tissue	20.3	11.1	27.8
5-2	Aortic root	21.5	12.1	28.9
6-1	Aortic root	25.5	11.7	16.0
6-2	Aortic root	26.6	12.2	17.0
7-1	Aortic root (coronary ostium)	10.8	16.0	9.7
7-2	Aortic root (valve leaflets inferior)	11.0	16.2	10.7
8-1	Aortic root	25.5	14.3	16.4
8-2	Aortic root	26.0	15.9	17.1
Average		**17.8**	**13.4**	**17.3**

Table 2.5: Average immunohistochemistry colour deconvolution results for the isolated aortic root aneurysm specimens.

2.6 Results

2.6.1 Demographics

In the ascending aortic aneurysm group, average age was 65.0 years and there were more males (n = 7) compared to females (n = 4). Reported medical comorbidities were hypertension (9/11), diabetes (3/11) and CVA (1/11). In the aortic root group, average age was 53.5 years and there were two males (n = 2) and one female (n = 1). One valve was bicuspid, and hypertension was the most reported comorbidity (2/3) (Table 2.1 and 2.2). In the non-aneurysmal cadaveric group, average age was 73.8 years and there was only one female in the group. Three patients died from cancer related complications, and two from respiratory related complications. Past medical histories were not known beyond the primary and secondary causes of death.

2.6.2 Observational analysis

Intimomedial tearing or extent of the dissection tear was variable amongst each patient depending on the origin of the tear and its extent of its propagation (Tables 2.6 and 2.7) (Fig. 2.1).

Patient	Intimomedial tear (dissecting aneurysm)	Insudation of plasma protein(PAS positive)/erythrocytes	Elastic fibre disruption/fragmentation/diminution	Medial fibrosis (increased collagen)	Thrombosis	Mineralisation (calcification)	Mural hyalinization	Mucoid degeneration	Chondroid metaplasia (cartilage deposition)	Cholesterol clefts	Additional features
Patient1	Proximal (HE)	Proximal (HE)	Proximal (HE) Distal (HE) Proximal (EVG)	Proximal (EVG) Proximal (massons)		Proximal (HE)	Proximal (HE)		Proximal (HE) Proximal (Massons)		**No elastin proximally**
Grade and distributions	Severe Extensive	Moderate and focal	Proximal/Distal mild Proximal (EVG) severe Extensive	Proximal EVG moderate Proximal (massons) Severe extensive		Moderate and extensive	Mild and focal		Proximal HE+ Proximal Massons moderate and extensive		
Patient 2	Proximal (anterior and posterior), middle (minor)										
Grade and distributions	Proximal moderate Distal moderate Middle mild Extensive										
Patient 3	Proximal (anterior and posterior) Middle (minor) – all stains							Proximal (anterior and posterior) – all stains Minor in middle posterior and distal (anterior)			
Grade and distributions	Moderate Extensive							Mild and focal			
Patient 4	Proximal, Middle, Distal (anterior/outer) – all stains	Proximal (inner)	Proximal (inner)			Middle (posterior) Distal (anterior)		Proximal (outer)			**Proximal – homogenous material Insudation of plasma proteins**
Grade and distributions	Moderate Extensive	Moderate and focal	Moderate Extensive			Mild and focal		Mild and focal			
Patient 5	Proximal – all sites	Proximal	Proximal – all sites	Proximal (inner and outer) Proximal (inner) – Massons	Proximal (all sites)	Proximal (posterior) Middle (inner/outer/anterior)				Proximal – all sites	
Grade and distributions	Mild Focal	Mild and focal	Mild Focal	Moderate and extensive	Mild extensive	Severe and extensive				Moderate and extensive	
Patient 6	Proximal (all sites) Distal (all sites)	Proximal	Proximal Distal – minor	Proximal Distal – minor				Proximal			**thick and fibrous intima proximally, recoil of the elastic fibres in the outer media**
Grade and distributions	Severe Extensive	Moderate and focal	Proximal severe Distal mild Extensive	Proximal Severe and extensive (massons) Moderate(HE) Distal Minor				Mild and focal			
Patient 7	Proximal (inner/outer) Prox,Mid,Distal (Von Kossa +ve) Middle/Distal (Alcian blue +ve)					Middle		Proximal (inner and outer) Middle (all sites)			**Abundant collagen in proximal**
Grade and distributions	Mild Von Kossa moderate Alcian blue moderate all sites Extensive					Mild and focal		Mild and focal			
Patient 8	Proximal	Proximal	Minimal elastin	Proximal Middle (nil elastin)		Middle					**Abundant collagen in proximal**
Grade and distributions	Moderate Extensive	Mild and focal		Moderate and extensive		Mild and focal					
Patient 9	Proximal (all sites)			Proximal, small distal	Proximal			Proximal			
Grade and distributions	Moderate Extensive			Moderate focal	Moderate and extensive			Moderate and extensive			
Patient 10	Proximal (inner and outer)	Proximal	Minimal elastin all regions	Proximal (masons)							
Grade and distributions	Moderate Extensive	Mild and focal		Moderate and extensive							
Patient 11	Proximal (inner)			Proximal (masons)						Proximal – all sites	**Abundant collagen proximally**
Grade and distributions	Moderate Extensive			Moderate and extensive						**Moderate and extensive**	

Table 2.6: Summary of observational analysis in aneurysmal patients *Boxes filled if not observed. Grade and distribution determined using standardised grading system (53)**

Patient	Intimomedial tear (dissecting aneurysm)	Elastic fibre disruption/fragmentation/diminution	Medial fibrosis	Thrombosis	Mineralisation (calcification)	Mural hyalinisation	Mucoid degeneration	Chondroid metaplasia (cartilage deposition)	Cholesterol clefts	Additional features
Patient1	Present in sinus tissue	Increased density of collagen	Abundant throughout	Not present	Not present	Not present	Present in sinus tissue	Not present	Not present	Increased density of collagen in adventitial and subintimal layers
Grade		Moderate and extensive	Moderate and extensive				Moderate and focal			
Patient 2	Present in sinus tissue	Increased density of collagen	Abundant throughout	Not present	Not present	Present in sinus tissue	Present in sinus tissue	Not present	Not present	Increased density of collagen in adventitial and subintimal layers
Grade	Moderate and focal	Moderate and extensive	Moderate and extensive				Moderate and focal			
Patient 3	Present in sinus tissue	Increased density of collagen	Abundant throughout	Not present	Not present	Not present	Not present	Not present	Not present	Increased density of collagen in adventitial Dense clumped vessels
Grade	Moderate and focal	Moderate and extensive	Moderate and extensive							

Table 2.7: Summary of observational analysis in aortic root aneurysm patients *Boxes filled if not observed.

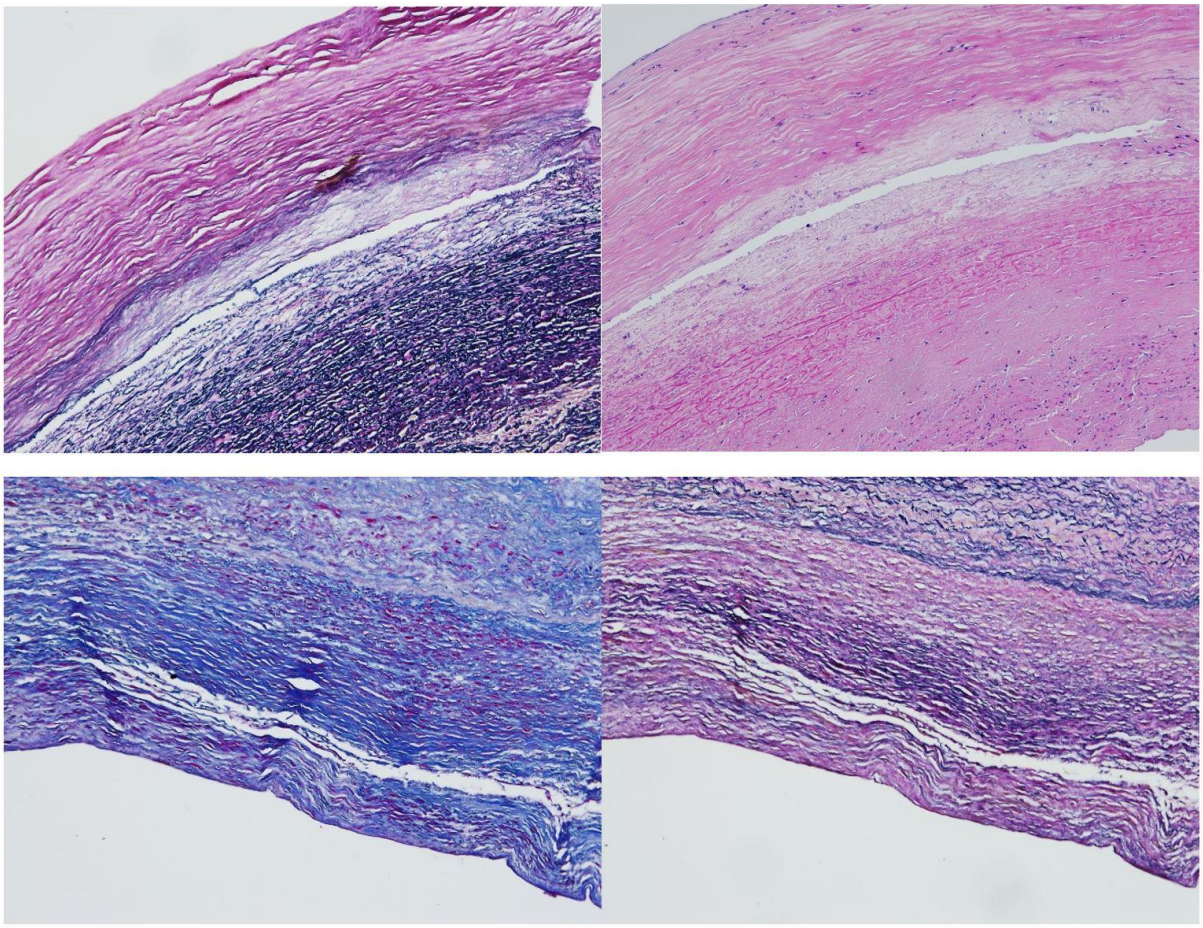

Figure 2.1: Ascending aorta aneurysm specimen pictures showing intimomedial tears or dissecting aneurysms (Top left (EVG) and Top right(H&E).) Aneurysms with Massons stain (Bottom left), and EVG aneurysm (Bottom right).

Ascending aorta aneurysm specimen pictures showing intimomedial tears or dissecting aneurysms (Top left (EVG) and Top right (H&E)). Aneurysms with Massons stain (Bottom left), and EVG aneurysm (Bottom right)

Elastic fibre fragmentation, medial fibrosis, thrombosis, and mural hyalinisation was greatest in the proximal aneurysmal ascending aorta (Fig. 2.2). Collagen density was increased in all aneurysmal specimens, confirmed by Von Kossa staining (Fig. 2.2). Mineralisation and calcification were greatest in the mid ascending aorta in aneurysmal samples. Mucoid degeneration was seen in the proximal aneurysmal regions (Fig. 2.3) and confirmed by Alcian blue staining.

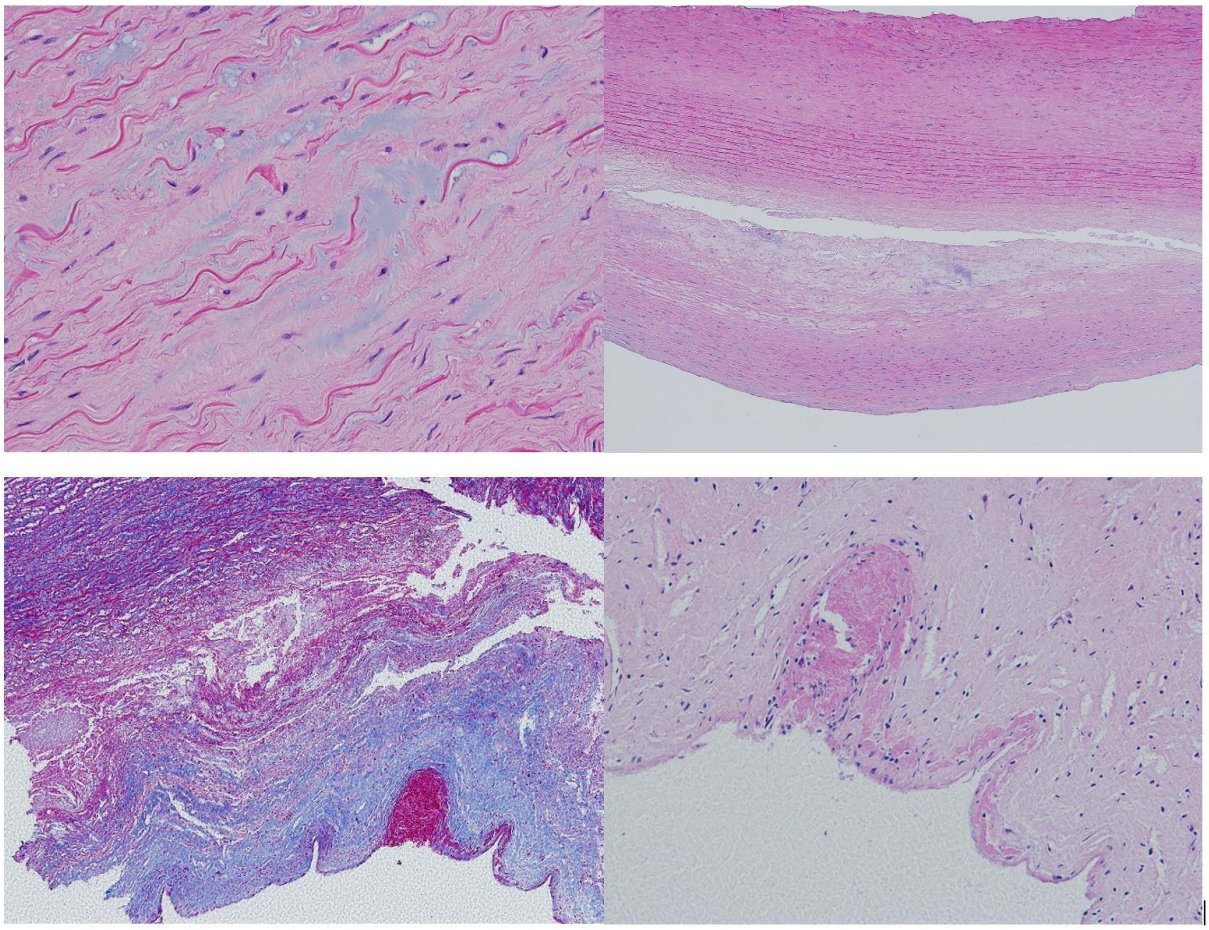

Figure 2.2: Elastic fibre disruption and fragmentation in H&E-stained segment of proximal ascending aorta aneurysm (Top left). Clear intimomedial tear with complete loss of elastin fibre structure and fibrosis in H&E-stained specimen (Top right). EVG (Bottom left) and H&E (Bottom right) stained images showing thrombosis present in the proximal regions of the ascending aortic aneurysm samples.

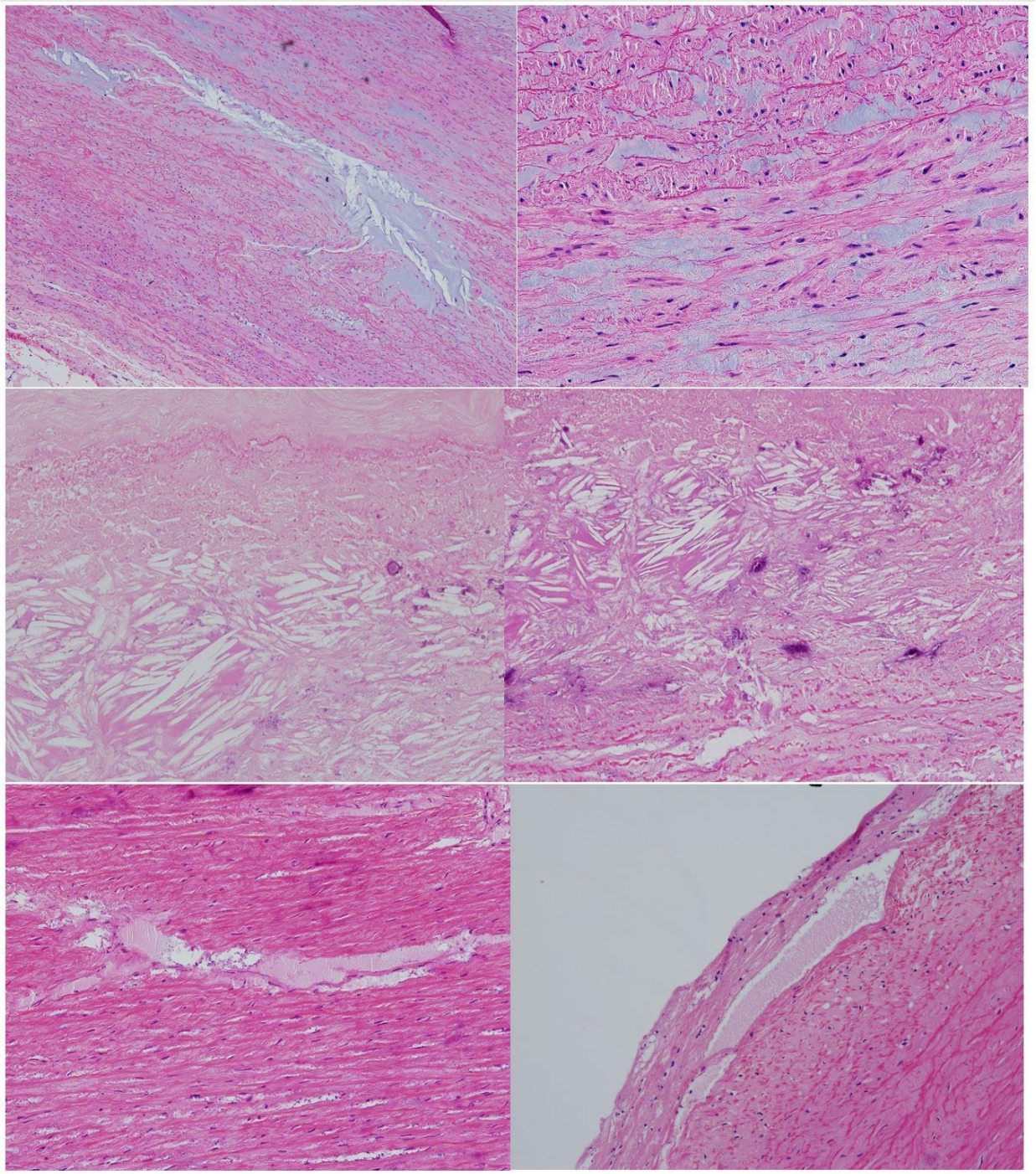

Figure 2.3: Mucoid degeneration around proximal ascending aorta aneurysm (Top left) and gross mucoid degeneration in H&E-stained aneurysmal sample (Top right). Cholesterol clefts in proximal ascending aorta specimens (Middle left and right). Protein insudation surrounding ascending aorta aneurysms in proximal regions. Seen in H&E images (Bottom left and right).

Chondroid metaplasia, cartilage deposition, protein insudation and cholesterol clefts was also observed to be greater in the proximal segments of the ascending aorta aneurysm specimens, and not present in the aortic root specimens. (Fig. 2.3).

The most significant additional findings found in the ascending aorta and aortic root aneurysm specimens, were the presence of high-density collagen fibres and lack of elastin fibres on observation. A summary of the basic histology observational findings in the aneurysmal groups is presented in the tables above.

Collagen I was seen in increased density throughout all regions of aneurysmal ascending aorta specimens, with positive blood vessel control, and in the media of the aneurysmal aortic root (Fig. 2.4). Minimal collagen I was seen in non-aneurysmal samples.

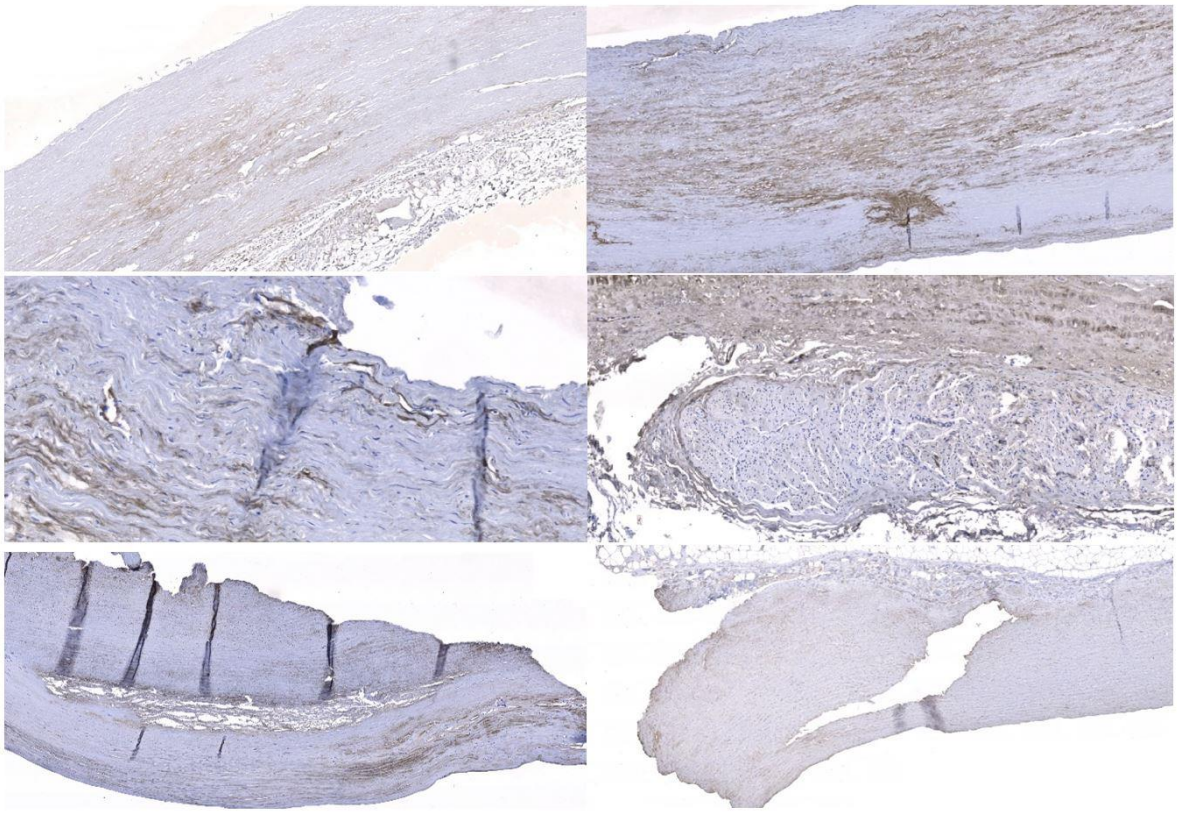

Figure 2.4: Increased density of Collagen I (brown staining) in all regions of the aorta, with increased density within the media (Top left and right). Collagen I images in ascending aorta aneurysms showing positive staining around vascular structures (Middle left and right). Collagen III images showing increased antibody uptake around intimal tears and generalised staining within the media (Bottom left and right).

Collagen III stained strongly in the media in most samples and around the areas of the intimal tearing (Fig. 2.4). Collagen III was distributed more evenly throughout the aortic root aneurysm samples. Collagen III was scarce in non-aneurysmal samples.

Collagen IV showed weak generalised staining throughout all ascending aorta aneurysm samples, with increased staining around the intimal tears and positive blood vessel controls (Fig. 2.5). Increased density of collagen and collagen clumping is seen in all aortic root aneurysm samples (Fig. 2.5). Collagen IV was scarce in the non-aneurysmal samples. A summary of observational analysis is shown in tables 2.8-2.11.

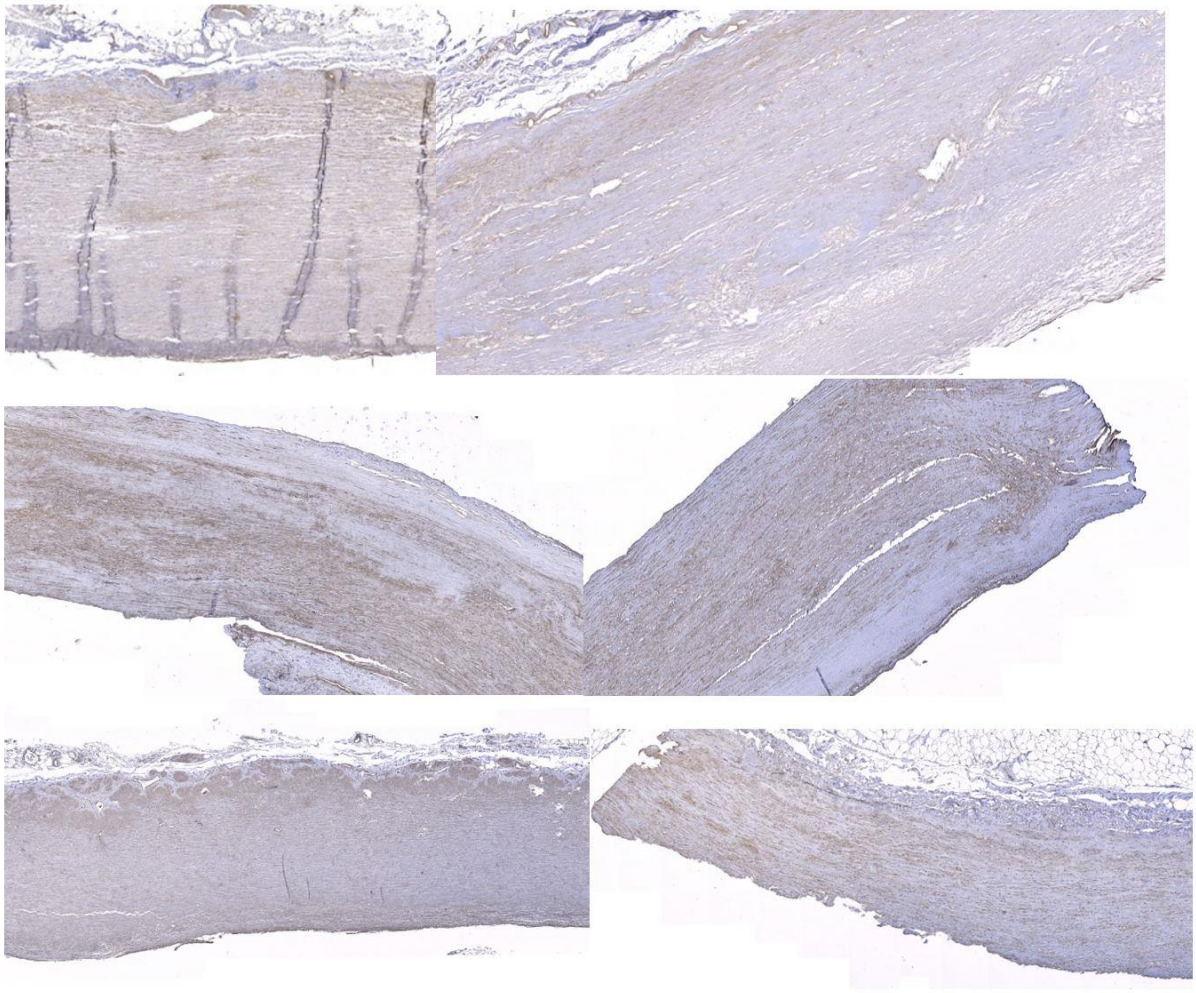

Figure 2.5: Generalised Collagen IV staining around ascending aorta aneurysm specimens with positive blood vessel controls (Top left and right). Collagen IV staining showing very generalised staining and increased staining around tears (Middle left and right). Collagen IV staining in the aortic root showing unique clumping of collagen very different to ascending aorta samples (Bottom left and right).

Patient	Intimomedial tear (dissecting aneurysm)	Insudation of plasma protein(PAS positive)/erythrocytes	Elastic fibre disruption/fragmentation/diminution	Medial fibrosis	Endothelium disruption/loss of integrity	Mineralisation (calcification)	Mural hyalinisation	Mucoid degeneration	Chondroid metaplasia (cartilage deposition)	Neovascularisation	Cholesterol clefts	Additional features
Patient1	Distal				Proximal	Middle						degenerative changes proximally
Grade					Mild and focal	Mild ad focal						
Patient 2				Proximal								
Grade				Mild and focal								
Patient 3			Proximal									
Grade			Moderate and focal									
Patient 4		Proximal	Proximal (inner) - fragmented						Proximal	Proximal	Proximal (inner)	
Grade		Mild and focal	Mild and focal						Mild and focal	Mild and focal	Mild and focal	
Patient 5			Distal - disrupted	Proximal			Proximal Distal					Increased fibrous tissue proximally
Grade			Mild and focal	Mild and focal			Mild and focal					
Patient 6		Proximal		Proximal				Proximal				
Grade		Mild and focal		Mild and focal				Mild and focal				
Patient 7												Nil significant findings
Grade												

Table 2.8: Summary of observational analysis in non-aneurysmal patients *Boxes filled if not observed.

Observations	Collagen I	Collagen III	Collagen IV
Patient1	• Increased density of collagen I in proximal, middle, distal • Positive control around blood vessel	• Strong staining around aneurysm and in media regions	• Weak staining in all regions
Patient 2	• Negative staining result	• Strong staining around aneurysm and in media regions	• Weak staining in all regions
Patient 3	• Increased density of collagen I in proximal, middle, distal • Positive control around blood vessel	• Strong staining around aneurysm and in media regions	• Increased collagen staining around split
Patient 4	• Increased density of collagen I in proximal, middle, distal • Positive control around blood vessel	• Strong staining around aneurysm and in media regions	• Increased collagen staining around split
Patient 5	• Increased density of collagen I in proximal, middle, distal • Positive control around blood vessel	• Strong staining around aneurysm and in media regions	• Increased collagen staining around split
Patient 6	• Increased density of collagen I in proximal, middle, distal • Positive control around blood vessel	• Strong staining around aneurysm and in media regions	• Increased collagen staining around split
Patient 7	• Negative result in middle region • Increased density of collagen I in proximal, distal • Positive control around blood vessel	• Strong staining around aneurysm and in media regions	• Increased collagen staining around split
Patient 8	• Negative result in middle region • Increased density of collagen I in proximal, distal • Positive control around blood vessel	• strong adventitial and intimal layers • less staining around split • minimal medial staining	• diffuse staining, strong staining around aneurysm in all regions
Patient 9	• Strong media and adventitia staining in proximal regions • Non-consistent staining	• Strong staining around aneurysm and in media regions	• Increased collagen staining around split
Patient 10	• Abundant collagen 1 staining throughout, more than control, greater in middle region	• more staining around aneurysm, strong staining around split in distal region • outer media strong staining, strong generalized staining in middle region • gross staining throughout, weaker staining in media, intima weaker, strong outer media in proximal region	• increased collagen staining around split, good internal control staining around vessels in proximal region
Patient 11	• Increased density of collagen I in proximal, middle, distal • Positive control around blood vessel	• Strong staining around aneurysm and in media regions	• Increased collagen staining around split

Table 2.9: Summary of immunohistochemistry observational analysis in aneurysmal patients.

Observations	Collagen I	Collagen III	Collagen IV
Patient1	• abundant collagen 1 in media, • adventitia increased density • multifocal subintimal density, strong adventitial, media	• generalised increased deposition in all layers • multifocal staining	• increased density of collagen, uniform, thick
Patient 2	• abundant collagen 1 in media, • adventitia increased density • multifocal subintimal density, strong adventitial, media	• generalised increased deposition in all layers • multifocal staining	• increased density of collagen, uniform, thick
Patient 3	• diffuse staining throughout • less in subintimal • positive control around blood vessels	• strong adventitial and intima, general media	• dense, clumped, vessels strongly positive

Table 2.10: Summary of immunohistochemistry observational analysis in aortic root aneurysm patients.

Observations	Collagen I	Collagen III	Collagen IV
Patient 1	• minimal collagen 1, normal distribution in distal regions • minimal collagen 1, normal distribution in middle regions	• no unique characteristics observed	• no unique characteristics observed
Patient 2	• subintimal staining intense, adventitial staining intense in distal regions	• no unique characteristics observed	• no unique characteristics observed
Patient 3	• standard medial density, adventitial density intense	• strong in adventitia and intima, less strong in media proximally	• strong intimal, adventitia, low medial density proximally
Patient 4	• normal collagen density distally	• minimal adventitial staining, dense stained intima proximally	• no unique characteristics observed
Patient 5	• no unique characteristics observed	• no unique characteristics observed	• no unique characteristics observed
Patient 6	• no unique characteristics observed	• no unique characteristics observed	• diffuse medially, strong intimal and adventitial proximally
Patient 7	• no unique characteristics observed	• no unique characteristics observed	• no unique characteristics observed

Table 2.11: Summary of immunohistochemistry observational analysis in non-aneurysmal patients.

2.6.3 Colour deconvolution results

Elastin content showed no clear pattern in aneurysmal versus non-aneurysmal samples. It was higher in aortic root aneurysms (Table 2.12) versus non-aneurysms (Table 2.13), and regionally highest in the inner parts (Table 2.14). Differences were not significantly different (p value = 0.20). (Fig. 2.6).

Specimen	Location	EVG results (%)	Massons trichrome results (%)
1-1	Aortic root sinus tissue	9.2	42.6
1-2	Aortic root sinus tissue	10.3	40.5
2-1	Aortic root sinus tissue	15.2	20.4
2-2	Aortic root sinus tissue	15.5	21.5
3-1	Aortic root sinus tissue	16.9	31.9
3-2	Aortic root sinus tissue	17.4	30.6
4-1	Aortic root sinus tissue	13.0	25.9
4-2	Aortic root sinus tissue	13.5	25.1
5-1	Aortic root sinus tissue (coronary ostia)	38.8	7.7
5-2	Aortic root sinus tissue (coronary ostia)	37.8	8.4
6-1	Aortic root sinus tissue	20.8	11.5
6-2	Aortic root sinus tissue	21.6	12.5
7-1	Aortic root sinus tissue (coronary ostia)	17.0	6.0
7-2	Aortic root sinus tissue (coronary ostia)	16.4	6.4
8-1	Aortic root sinus tissue	27.4	6.5

8-2	Aortic root sinus tissue	28.4	6.4
9-1	Aortic root sinus tissue	23.7	14.7
9-2	Aortic root sinus tissue	25.0	15.5
10-1	Aortic root sinus tissue	30.4	11.2
10-2	Aortic root sinus tissue	31.5	11.6
11-1	Aortic root sinus tissue	30.7	8.8
11-2	Aortic root sinus tissue	31.5	9.4
12-1	Aortic root sinus tissue	43.9	11.9
12-2	Aortic root sinus tissue	42.5	12.6
13-1	Aortic root sinus tissue (valvular tissue inferior)	6.1	7.5
14-1	Aortic root sinus tissue (valvular tissue inferior)	7.2	8.4
15-1	Aortic root sinus tissue (valvular tissue inferior)	8.2	13.4
16-1	Aortic root sinus tissue (valvular tissue inferior)	9.4	14.6
17-1	Aortic root sinus tissue	13.1	28.2
18-1	Aortic root sinus tissue	14.2	29.5
19-1	Aortic root sinus tissue	14.4	15.7
20-1	Aortic root sinus tissue	15.7	14.6
Average		**20.8**	**16.6**

Table 2.12: Summary of the colour deconvolution results from the aortic root aneurysm patients.

Patient	**Proximal (%)**				**Middle (%)**				**Distal (%)**				**Root (%)**
	Anterior	**Posterior**	**Inner**	**Outer**	**Anterior**	**Posterior**	**Inner**	**Outer**	**Anterior**	**Posterior**	**Inner**	**Outer**	
1-1	8.4	9.5	14.6	7.0	6.5	11.9	18.6	26.2	5.8	9.4	15.2	20.4	10.6
1-2	9.2	10.5	14.0	11.5	6.7	15.6	17.5	24.6	9.4	12.0	20.9	19.7	11.5
2-1	15.1	13.8	12.8	23.8	11.7	14.4	17.2	23.2	11.9	10.1	25.0	21.2	15.7
2-2	12.4	19.5	17.7	26.9	8.5	14.6	16.3	15.0	13.0	11.5	23.7	22.0	16.3
3-1	10.3	12.3	12.3	11.3	12.3	10.6	25.9	17.2	15.9	14.6	23.3	13.4	20.6
3-2	9.5	13.0	12.0	12.0	14.3	14.2	32.1	17.2	18.6	22.6	16.9	16.2	19.3
4-1	3.5	6.3	4.9	6.1	7.3	5.7	4.9	15.0	11.4	11.3	22.3	20.4	16.4
4-2	4.1	6.5	5.0	6.1	7.3	5.5	4.1	15.4	12.1	10.2	21.3	22.1	15.6
5-1	12.9	11.1	22.3	15.1	16.7	16.8	39.3	19.7	28.4	21.9	29.0	21.5	11.5
5-2	11.0	11.9	22.9	15.8	16.3	16.5	35.6	19.5	27.4	21.5	28.5	21.5	10.9
6-1	8.4	11.5	12.5	11.6	12.5	11.0	18.5	24.0	10.0	11.5	24.1	20.5	20.0
6-2	8.6	10.3	12.1	10.6	12.5	14.9	18.5	23.8	9.5	11.5	24.8	20.5	23.2
7-1	9.6	11.5	11.5	11.5	11.0	15.5	19.2	22.9	12.6	10.5	23.5	21.5	20.5
7-2	9.5	11.5	11.1	10.3	11.4	16.5	19.0	23.9	11.6	10.5	24.0	20.5	22.0
Average	**9.5**	**11.4**	**13.3**	**12.8**	**11.1**	**13.1**	**20.5**	**20.6**	**14.1**	**13.5**	**23.0**	**20.1**	**16.7**
Standard deviation	***3.08***	***3.18***	***5.17***	***6.06***	***3.40***	***3.75***	***10.04***	***3.99***	***6.56***	***4.76***	***3.74***	***2.42***	***4.33***

Table 2.13: Summary of colour deconvolution analysis in elastic tissue composition via EVG staining in non-aneurysmal patients.

Patient	Proximal (%)				Middle (%)				Distal (%)			
	Anterior	Posterior	Inner	Outer	Anterior	Posterior	Inner	Outer	Anterior	Posterior	Inner	Outer
1 -1	9.3	6.7	18.5	10.2	17.2	12.5	23.1	25.7	11.7	14.6	21.0	15.8
1-2	10.3	7.2	19.6	12.1	20.8	8.8	24.9	24.0	15.5	13.6	21.0	15.8
2-1	24.7	14.7	24.7	11.3	14.6	17.2	12.7	14.7	15.9	18.5	6.5	10.9
2-2	23.0	12.6	22.5	12.3	14.0	15.7	10.7	12.7	17.0	15.5	8.5	12.0
3-1	12.4	13.5	23.7	13.5	12.0	20.2	29.8	32.2	25.8	23.3	28.6	14.4
3-2	11.8	11.4	23.0	12.4	12.0	19.0	28.3	28.9	25.8	20.4	17.8	16.1
4-1	17.4	7.3	14.4	13.5	14.5	10.9	16.4	10.1	11.6	11.6	23.4	21.4
4-2	14.4	9.5	9.8	15.9	11.1	11.0	15.9	10.6	9.1	11.6	21.4	20.4
5-1	21.3	9.7	12.2	25.1	9.2	15.5	27.1	17.0	11.1	11.6	23.4	21.4
5-2	23.1	8.7	12.1	21.3	9.3	15.5	24.5	22.6	13.0	16.0	23.4	20.6
6-1	8.8	8.8	17.7	13.8	8.8	20.2	13.5	11.8	22.9	20.3	23.3	16.6
6-2	9.9	9.1	19.3	13.9	15.8	21.3	23.8	13.5	20.4	21.0	30.5	16.5
7-1	22.1	23.0	21.9	10.3	19.9	14.0	11.6	16.6	19.3	15.0	19.2	14.3
7-2	22.4	23.3	21.1	12.1	17.1	13.9	18.2	16.7	17.3	10.1	25.1	27.5
8-1	8.9	10.5	19.0	5.7	22.8	11.0	19.6	21.4	19.0	11.2	13.2	19.2
8-2	8.8	11.5	21.4	8.9	23.7	11.9	20.7	22.2	19.0	12.2	13.8	20.1
9-1	9.4	8.5	19.0	7.1	15.8	6.1	12.3	13.0	24.7	11.2	13.2	10.0
9-2	9.9	12.0	19.0	8.4	14.2	8.7	18.8	13.0	19.4	12.5	10.6	12.9
10-1	9.4	6.5	15.3	14.8	11.7	12.5	19.6	12.8	8.3	19.5	14.0	13.4
10-2	9.1	7.3	12.6	17.7	10.6	16.8	12.7	8.1	7.6	20.4	10.3	15.6
11-1	11.4	6.9	12.4	10.8	9.6	10.5	18.6	9.7	6.3	12.7	13.1	12.5
11-2	11.1	7.3	12.6	10.7	10.6	10.8	18.7	8.1	7.6	12.4	12.3	13.6
Average	**14.0**	**10.7**	**17.8**	**12.8**	**14.3**	**13.8**	**19.2**	**16.6**	**15.8**	**15.2**	**17.9**	**16.4**
Standard deviation	***5.85***	***4.64***	***4.42***	***4.39***	***4.42***	***4.11***	***5.64***	***6.86***	***6.13***	***4.03***	***6.70***	***4.23***

Table 2.14: Summary of colour deconvolution analysis in elastic tissue composition via EVG staining in aneurysmal patients.

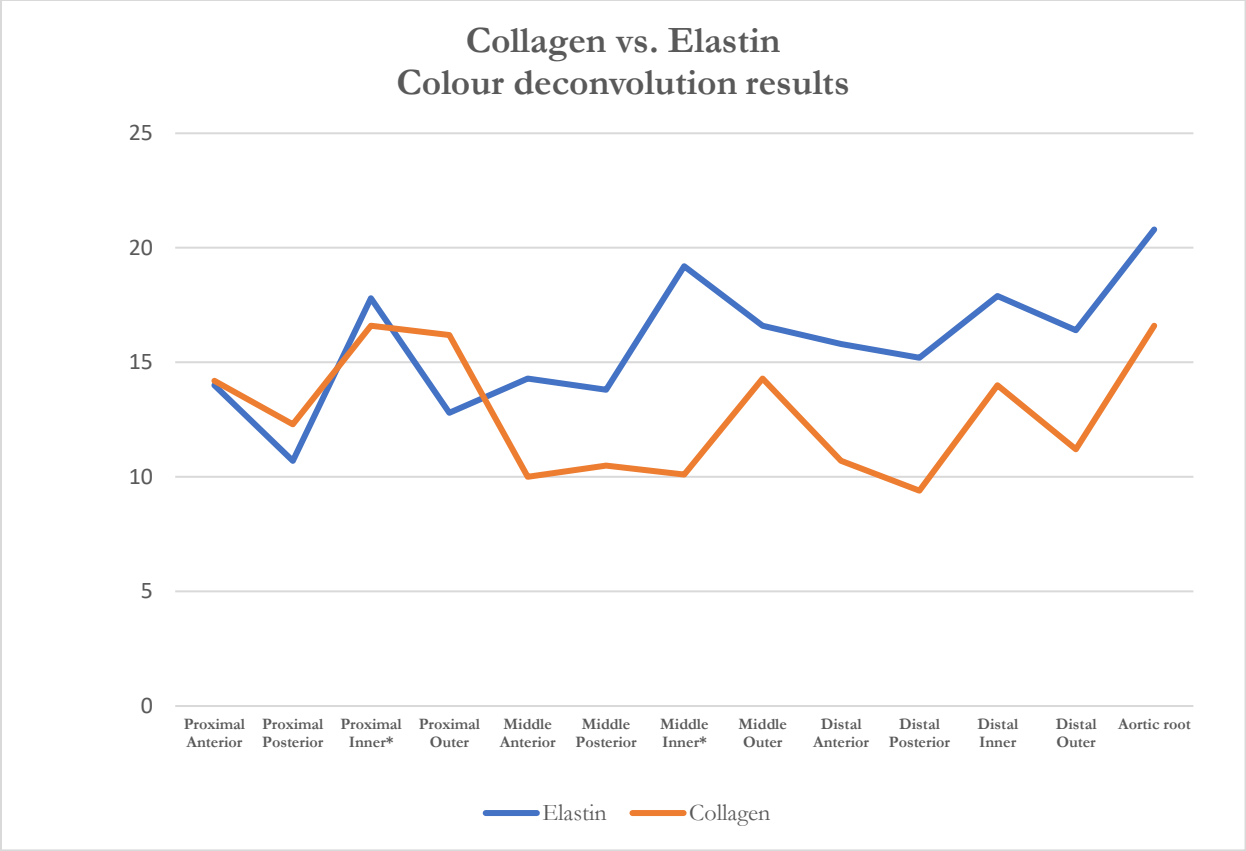

Figure 2.6: Colour deconvolution comparison between collagen and elastin in aneurysmal ascending aorta and aortic root regions. *denotes regions of statistical significance.

Collagen content was clearly higher in proximal ascending aorta aneurysms (Table 2.15) versus non-aneurysmal and other regions (p value = 0.0004), as well as higher in aortic root aneurysms (Table 2.12) versus non-aneurysmal samples (Table 2.16) (p-value = 0.00029).

Patient	Proximal (%)				Middle (%)				Distal (%)			
	Anterior	Posterior	Inner	Outer	Anterior	Posterior	Inner	Outer	Anterior	Posterior	Inner	Outer
1-1	6.6	2.3	12.6	9.2	2.1	5.4	3.7	1.9	9.2	15.4	6.5	10.2
1-2	9.1	2.9	14.6	6.4	2.1	5.4	4.1	2.0	11.8	13.5	7.8	8.5
2-1	11.1	18.3	15.5	9.6	19.5	15.4	8.1	13.3	16.2	21.8	14.3	15.3
2-2	13.3	19.0	14.6	10.6	17.5	14.4	10.1	14.1	16.0	20..0	12.2	13.0
3-1	6.9	6.9	28.0	12.1	9.4	7.8	4.3	16.1	21.9	5.0	22.7	9.8
3-2	5.1	5.2	23.8	15.1	9.4	5.8	5.0	15.1	15.1	4.2	22.7	9.7
4-1	19.8	2.9	10.3	9.0	25.1	23.4	12.2	13.0	16.2	5.9	21.2	9.9
4-2	16.4	4.1	12.2	10.0	22.7	20.6	12.7	12.9	16.1	6.9	21.9	10.0
5-1	10.5	13.7	12.0	11.1	1.4	0.7	14.5	3.5	2.1	14.0	12.2	8.1
5-2	15.3	13.7	10.9	10.5	1.4	0.9	12.5	2.6	1.7	16.1	12.1	7.1
6-1	9.1	16.9	20.0	18.0	7.2	0.8	15.7	12.2	3.6	3.1	23.3	15.4

6-2	7.3	11.6	22.0	17.9	8.1	0.9	19.6	17.9	3.0	3.1	28.5	13.4
7-1	25.1	14.6	21.1	16.4	26.1	19.9	20.4	23.1	14.6	12.6	11.3	22.5
7-2	23.1	15.0	22.0	17.1	26.9	19.1	20.7	23.0	15.4	12.7	13.2	21.2
8-1	17.2	22.2	16.7	19.3	5.3	13.4	10.1	20.6	3.5	3.5	17.4	11.1
8-2	17.7	22.3	17.6	20.0	7.7	14.6	11.1	21.4	5.7	5.7	18.9	12.3
9-1	17.8	16.3	16.7	16.9	5.3	16.8	10.1	20.6	2.9	2.9	16.7	6.0
9-2	20.5	16.9	19.3	16.9	5.3	20.2	10.1	21.5	3.5	2.9	12.9	4.9
10-1	18.6	11.8	13.3	28.6	4.6	7.1	4.1	16.7	15.4	12.9	2.3	9.4
10-2	12.2	9.0	14.3	28.6	4.5	6.1	4.0	14.8	11.8	11.7	2.1	10.1
11-1	15.6	11.0	13.9	26.0	5.4	7.1	4.1	14.6	14.6	12.0	3.3	9.5
11-2	14.8	13.2	14.7	28.1	4.1	6.1	4.2	14.7	14.1	12.3	4.1	9.2
Average	**14.2**	**12.3**	**16.6**	**16.2**	**10.0**	**10.5**	**10.1**	**14.3**	**10.7**	**9.4**	**14.0**	**11.2**
Standard deviation	***5.58***	***6.15***	***4.62***	***6.78***	***8.60***	***7.39***	***5.60***	***6.63***	***6.21***	***5.51***	***7.59***	***4.32***

Table 2.15: Summary of colour deconvolution analysis in collagen tissue composition via Massons trichrome staining in aneurysmal patients.

Patient	**Proximal (%)**				**Middle (%)**				**Distal (%)**				**Root (%)**
	Anterior	**Posterior**	**Inner**	**Outer**	**Anterior**	**Posterior**	**Inner**	**Outer**	**Anterior**	**Posterior**	**Inner**	**Outer**	
1-1	14.7	6.5	6.0	15.6	7.6	3.6	6.7	7.5	9.5	5.9	8.7	14.4	6.5
1-2	13.5	7.4	7.3	14.3	17.8	4.9	5.7	8.5	14.3	5.1	8.6	28.3	14.4
2-1	9.1	15.1	9.2	17.5	12.4	16.6	5.3	14.7	3.7	4.3	7.1	18.1	15.4
2-2	10.5	14.5	9.6	16.6	12.0	15.6	6.5	13.9	4.5	5.4	8.0	17.3	13.4
3-1	17.8	9.4	9.5	9.6	12.9	6.8	7.7	13.9	9.6	5.7	8.4	15.3	9.1
3-2	15.5	8.1	10.0	10.5	13.5	7.5	7.4	13.3	10.4	6.4	8.2	15.4	9.6
4-1	3.6	7.0	2.2	3.4	1.0	1.3	0.5	2.7	1.7	0.8	0.8	1.5	4.4
4-2	3.6	6.5	3.5	3.5	1.5	2.0	1.5	3.4	2.4	1.3	1.8	1.9	3.4
5-1	3.6	7.0	2.2	3.4	1.0	1.3	0.5	2.7	1.7	0.8	0.8	1.5	7.5
5-2	4.8	5.5	3.5	3.6	1.5	2.0	0.8	2.8	1.5	0.9	1.5	1.6	6.3
6-1	10.5	8.3	6.5	15.3	7.2	4.7	5.0	7.8	4.6	5.7	8.9	14.1	12.5
6-2	12.0	8.2	5.4	15.3	7.0	5.0	5.4	8.2	4.1	5.1	7.2	14.3	10.2
7-1	9.6	6.0	7.4	14.0	7.1	4.1	5.5	7.3	3.0	6.4	8.3	13.2	12.6
7-2	8.5	6.3	7.3	12.0	5.5	4.4	6.0	6.4	4.4	6.4	7.3	13.0	10.6
Average	**9.8**	**8.3**	**6.4**	**11.1**	**7.7**	**5.7**	**4.6**	**8.1**	**5.4**	**4.3**	**6.1**	**12.1**	**9.7**
Standard deviation	***4.65***	***2.96***	***2.71***	***5.43***	***5.38***	***4.78***	***2.65***	***4.37***	***3.96***	***2.27***	***3.26***	***7.86***	***3.74***

Table 2.16: Summary of colour deconvolution analysis in collagen tissue composition via Massons trichrome staining in non-aneurysmal patients.

2.6.4 Immunohistochemistry histological analysis

Collagen I content was low in non-aneurysmal samples (Table 2.17), and high in aneurysmal aortic root specimens, particularly in the sinus tissue regions of the root structure (Table 2.18) (p value = 0.0005). Aneurysmal results are presented in Table 2.19 (Fig. 2.7).

Patient	Tissue samples	Distal (%)				Middle (%)				Proximal (%)				Root (%)
		Anterior	Posterior	Inner	Outer	Anterior	Posterior	Inner	Outer	Anterior	Posterior	Inner	Outer	Root
1(1)	Ascending aorta	12.4	13.0	10.3	9.8	9.4	6.5	11.7	4.4	8.9	11.5	3.5	2.3	8.5
1(2)	Ascending aorta	11.2	12.5	10.1	10.5	9.6	7.0	11.7	5.5	9.5	10.9	4.0	2.4	9.3
2(1)	Ascending aorta	9.5	14.5	15.4	8.9	13.9	11.8	12.4	18.5	18.7	11.0	11.5	19.4	13.5
2(2)	Ascending aorta	9.5	14.3	16.4	9.2	14.0	10.3	14.9	17.9	18.6	11.5	10.3	20.5	14.0
3(1)	Ascending aorta	18.5	15.5	15.3	11.7	19.4	10.2	12.2	10.2	8.8	17.6	19.5	14.1	15.4
3(2)	Ascending aorta	17.1	14.1	15.0	11.1	19.2	11.4	13.3	10.9	9.4	17.4	20.3	13.1	14.3
4(1)	Ascending aorta	20.7	15.3	14.9	15.8	12.9	18.1	17.2	17.7	20.3	12.1	16.1	14.4	20.5
4(2)	Ascending aorta	21.4	16.4	15.2	16.3	12.3	20.2	16.2	19.3	20.5	15.2	18.3	15.4	21.0
5(1)	Ascending aorta	12.4	21.3	27.0	12.5	5.7	2.4	17.5	21.4	8.5	6.2	14.8	5.4	9.3
5(2)	Ascending aorta	11.3	20.6	25.9	12.5	6.2	3.2	15.4	20.2	8.6	7.3	15.5	6.6	10.3
6(1)	Ascending aorta	12.7	15.6	15.5	10.3	14.7	7.6	15.5	22.8	10.0	11.5	17.3	14.6	15.8
6(2)	Ascending aorta	14.2	16.5	15.9	11.0	13.6	8.5	15.8	21.5	9.9	11.0	17.3	12.4	14.5
7(1)	Ascending aorta	11.3	16.4	19.3	9.5	15.2	11.3	14.3	25.5	13.3	11.3	19.0	9.8	14.4
7(2)	Ascending aorta	10.2	16.1	18.5	9.6	15.6	10.3	14.3	23.5	14.4	10.0	19.3	8.9	16.3
Average		**13.8**	**15.9**	**16.8**	**11.4**	**13.0**	**9.9**	**14.5**	**17.1**	**12.8**	**11.7**	**14.8**	**11.4**	**14.1**

Table 2.17: Collagen I analysis via colour deconvolution in non-aneurysmal patients.

Specimen number	Tissue region	Collagen I (%)	Collagen III (%)	Collagen IV (%)
1-1	Aortic root sinus tissue	22.5	14.3	15.6
1-2	Aortic root sinus tissue	23.5	15.2	16.3
2-1	Aortic root sinus tissue (coronary ostium)	8.2	10.9	21.1
2-2	Aortic root sinus tissue (coronary ostium)	9.2	11.3	22.5

3-1	Aortic root sinus tissue (valve leaflets inferiorly)	10.5	16.7	17.7
3-2	Aortic root sinus tissue	11.5	18.5	19.5
4-1	Aortic root sinus tissue	15.7	8.8	9.9
4-2	Aortic root sinus tissue	16.5	9.5	10.5
5-1	Aortic root sinus tissue	20.3	11.1	27.8
5-2	Aortic root	21.5	12.1	28.9
6-1	Aortic root	25.5	11.7	16.0
6-2	Aortic root	26.6	12.2	17.0
7-1	Aortic root (coronary ostium)	10.8	16.0	9.7
7-2	Aortic root (valve leaflets inferior)	11.0	16.2	10.7
8-1	Aortic root	25.5	14.3	16.4
8-2	Aortic root	26.0	15.9	17.1
Average		**17.8**	**13.4**	**17.3**

Table 2.18: Average immunohistochemistry colour deconvolution results for the isolated aortic root aneurysm specimens.

Patient	Tissue samples	Distal (%)				Middle (%)				Proximal (%)			
		Anterior	Posterior	Inner	Outer	Anterior	Posterior	Inner	Outer	Anterior	Posterior	Inner	Outer
1(1)	Ascending aorta	2.4	6.3	3.3	10.4	5.4	7.3	10.5	9.3	2.2	6.4	6.6	8.1
1(2)	Ascending aorta	2.7	5.3	3.7	10.4	6.3	8.0	9.4	10.0	2.4	6.1	5.4	8.4
2(1)	Ascending aorta	16.9	16.5	18.0	5.8	6.8	10.4	4.1	7.0	3.5	8.0	6.9	8.0
2(2)	Ascending aorta	16.5	16.2	17.4	6.6	5.4	10.6	4.0	6.8	4.5	8.4	7.9	7.4
3(1)	Ascending aorta	3.4	11.4	6.2	3.7	9.7	7.6	3.5	5.8	5.4	5.0	3.9	4.1
3(2)	Ascending aorta	3.4	10.5	6.0	3.8	10.4	7.5	3.6	5.5	5.4	4.6	3.2	4.1
4(1)	Ascending aorta	11.3	5.5	9.5	5.1	6.0	2.9	4.1	6.0	1.9	2.1	1.4	5.8
4(2)	Ascending aorta	10.4	4.4	10.6	4.6	6.4	3.3	4.6	6.4	2.5	3.3	2.5	6.3
5(1)	Ascending aorta	3.6	12.0	4.3	4.1	4.3	2.7	3.4	5.5	2.6	14.3	3.1	9.1
5(2)	Ascending aorta	3.6	10.5	5.3	4.6	5.6	3.5	4.4	6.0	2.5	13.4	3.5	9.3
6(1)	Ascending aorta	11.7	14.3	9.7	7.3	12.4	8.5	6.0	11.7	6.3	13.3	30.8	4.0

6(2)	Ascending aorta	10.5	13.6	10.4	6.4	12.4	9.3	6.5	10.3	3.4	12.4	28.3	4.5
7(1)	Ascending aorta	3.4	1.3	2.6	4.7	11.6	9.1	7.0	3.9	5.3	8.4	7.5	7.0
7(2)	Ascending aorta	3.2	2.9	3.5	5.2	11.6	9.0	7.9	4.2	5.0	8.3	6.3	7.8
Average		**7.3**	**9.3**	**7.9**	**5.9**	**8.2**	**7.1**	**5.6**	**7.0**	**3.8**	**8.1**	**8.4**	**6.7**

Table 2.19: Collagen I analysis via colour deconvolution in aneurysmal patients.

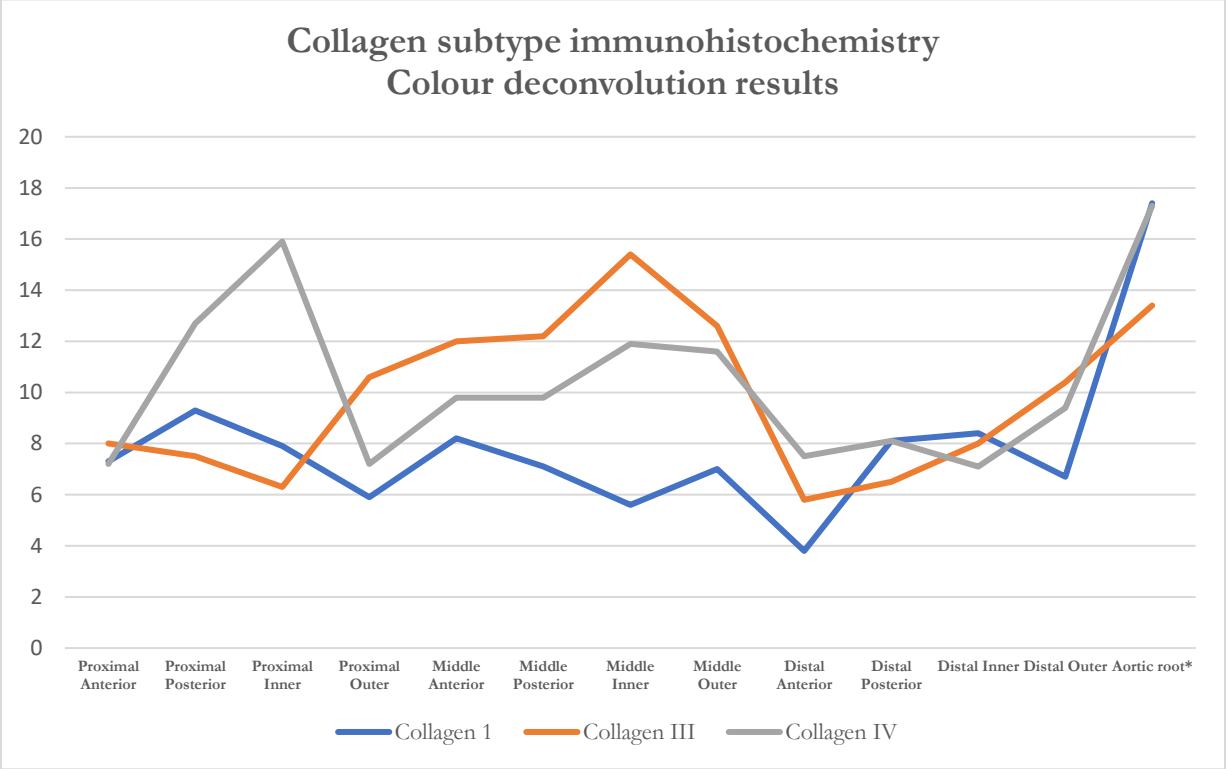

Figure 2.7: Colour deconvolution comparison between collagen subtypes in aneurysmal ascending aorta and aortic root regions. *denotes regions of statistical significance.

Collagen III content was lowest in the proximal region, and highest in the inner regions in aneurysmal ascending aorta patients (Table 2.20) (Figure 2.7), but no difference was observed between root regions (Table 2.21) p value = 0.44). Non-aneurysmal results are presented in Table 2.22.

Patient	Tissue samples	Distal (%)				Middle (%)				Proximal (%)			
		Anterior	Posterior	Inner	Outer	Anterior	Posterior	Inner	Outer	Anterior	Posterior	Inner	Outer
1(1)	Ascending aorta	10.6	7.1	6.2	10.4	26.8	33.8	19.7	24.3	5.9	5.5	5.1	12.1

1(2)	Ascending aorta	10.1	6.6	5.3	10.4	26.6	30.4	20.5	22.5	4.5	4.0	5.0	11.4
2(1)	Ascending aorta	10.6	7.0	12.7	13.4	14.1	8.5	15.0	7.6	2.6	3.9	1.6	3.6
2(2)	Ascending aorta	10.4	7.4	12.4	13.6	13.3	7.1	14.4	6.1	3.7	3.6	2.5	3.6
3(1)	Ascending aorta	6.8	7.5	2.7	7.0	8.9	8.6	12.5	5.6	3.3	3.2	5.4	3.0
3(2)	Ascending aorta	6.3	7.4	3.6	7.4	10.4	8.4	11.4	6.2	4.4	3.6	4.4	3.5
4(1)	Ascending aorta	6.8	2.9	5.6	10.7	3.1	5.1	3.6	7.5	8.3	5.8	7.9	6.1
4(2)	Ascending aorta	6.4	3.4	5.4	9.3	4.6	6.3	4.6	7.4	8.4	5.5	6.5	6.5
5(1)	Ascending aorta	6.5	5.5	5.9	13.7	8.1	7.4	21.2	8.9	7.4	15.3	7.0	17.6
5(2)	Ascending aorta	7.3	6.2	6.0	9.5	9.4	6.4	19.5	8.0	5.3	13.4	7.8	15.3
6(1)	Ascending aorta	10.9	9.1	6.0	10.4	15.9	20.3	15.8	17.7	5.3	7.3	21.5	18.2
6(2)	Ascending aorta	10.3	10.3	6.5	11.2	14.9	19.5	15.3	15.2	5.2	9.4	20.4	16.3
7(1)	Ascending aorta	5.3	12.2	5.2	10.5	13.6	15.7	24.7	26.3	8.9	4.6	6.5	15.3
7(2)	Ascending aorta	5.9	11.4	5.2	10.2	13.4	15.4	22.3	25.4	7.4	4.4	5.1	15.2
Average		**8.0**	**7.5**	**6.3**	**10.6**	**12.0**	**12.2**	**15.4**	**12.6**	**5.8**	**6.5**	**8.0**	**10.4**

Table 2.20: Collagen III analysis via colour deconvolution in aneurysmal patients.

Specimen number	Tissue region	Collagen I (%)	Collagen III (%)	Collagen IV (%)
1-1	Aortic root sinus tissue	22.5	14.3	15.6
1-2	Aortic root sinus tissue	23.5	15.2	16.3
2-1	Aortic root sinus tissue (coronary ostium)	8.2	10.9	21.1
2-2	Aortic root sinus tissue (coronary ostium)	9.2	11.3	22.5
3-1	Aortic root sinus tissue (valve leaflets inferiorly)	10.5	16.7	17.7
3-2	Aortic root sinus tissue	11.5	18.5	19.5
4-1	Aortic root sinus tissue	15.7	8.8	9.9
4-2	Aortic root sinus tissue	16.5	9.5	10.5
5-1	Aortic root sinus tissue	20.3	11.1	27.8
5-2	Aortic root	21.5	12.1	28.9
6-1	Aortic root	25.5	11.7	16.0
6-2	Aortic root	26.6	12.2	17.0
7-1	Aortic root (coronary ostium)	10.8	16.0	9.7
7-2	Aortic root (valve leaflets inferior)	11.0	16.2	10.7
8-1	Aortic root	25.5	14.3	16.4
8-2	Aortic root	26.0	15.9	17.1
Average		**17.8**	**13.4**	**17.3**

Table 2.21: Average immunohistochemistry colour deconvolution results for the isolated aortic root aneurysm specimens.

Patient	Tissue samples	Distal (%)				Middle (%)				Proximal (%)				Root (%)
		Anterior	Posterior	Inner	Outer	Anterior	Posterior	Inner	Outer	Anterior	Posterior	Inner	Outer	Root
1(1)	Ascending aorta	12.8	7.7	22.7	14.1	13.2	15.7	11.5	8.8	8.0	12.3	15.6	14.1	14.3
1(2)	Ascending aorta	10.7	8.4	21.5	14.1	13.1	15.9	11.4	8.9	7.2	12.3	15.0	13.5	14.3
2(1)	Ascending aorta	15.3	15.7	23.4	15.8	6.4	7.8	23.4	18.0	18.1	9.5	13.3	11.9	16.2
2(2)	Ascending aorta	16.4	15.4	20.6	14.2	7.4	7.9	22.0	19.2	19.0	10.4	15.6	13.3	16.3
3(1)	Ascending aorta	15.7	11.4	12.1	14.4	10.5	14.5	13.0	16.6	19.5	32.4	31.0	21.1	28.3
3(2)	Ascending aorta	14.5	12.0	13.0	13.6	11.4	15.0	14.3	15.3	19.5	30.9	30.2	23.4	30.4
4(1)	Ascending aorta	16.1	12.9	10.7	19.1	12.7	19.0	8.2	17.1	13.0	26.6	13.8	18.6	17.3
4(2)	Ascending aorta	15.4	12.6	11.4	20.1	13.2	17.6	9.5	16.3	13.7	25.3	14.5	17.7	16.4
5(1)	Ascending aorta	23.4	18.0	12.7	22.8	15.4	27.5	16.8	9.2	16.3	12.0	19.1	24.2	17.4
5(2)	Ascending aorta	22.5	17.4	14.5	23.5	16.6	25.3	16.3	10.4	15.4	13.7	19.2	23.6	19.5
6(1)	Ascending aorta	12.6	13.5	20.4	15.5	12.3	15.9	11.3	9.5	14.5	12.6	15.3	14.5	20.6
6(2)	Ascending aorta	13.2	15.0	21.5	14.1	12.5	15.2	12.0	8.9	16.4	11.0	14.6	13.5	15.4
7(1)	Ascending aorta	12.1	11.5	15.4	16.3	12.6	14.0	12.0	9.0	12.5	13.5	16.3	15.5	15.4
7(2)	Ascending aorta	12.3	12.1	15.7	15.1	12.5	16.5	12.9	10.4	13.0	14.4	16.3	14.4	16.0
Average		**15.2**	**13.1**	**16.8**	**16.6**	**12.1**	**16.3**	**13.9**	**12.7**	**14.7**	**16.9**	**17.9**	**17.1**	**18.4**

Table 2.22: Collagen III analysis via colour deconvolution in non-aneurysmal patients.

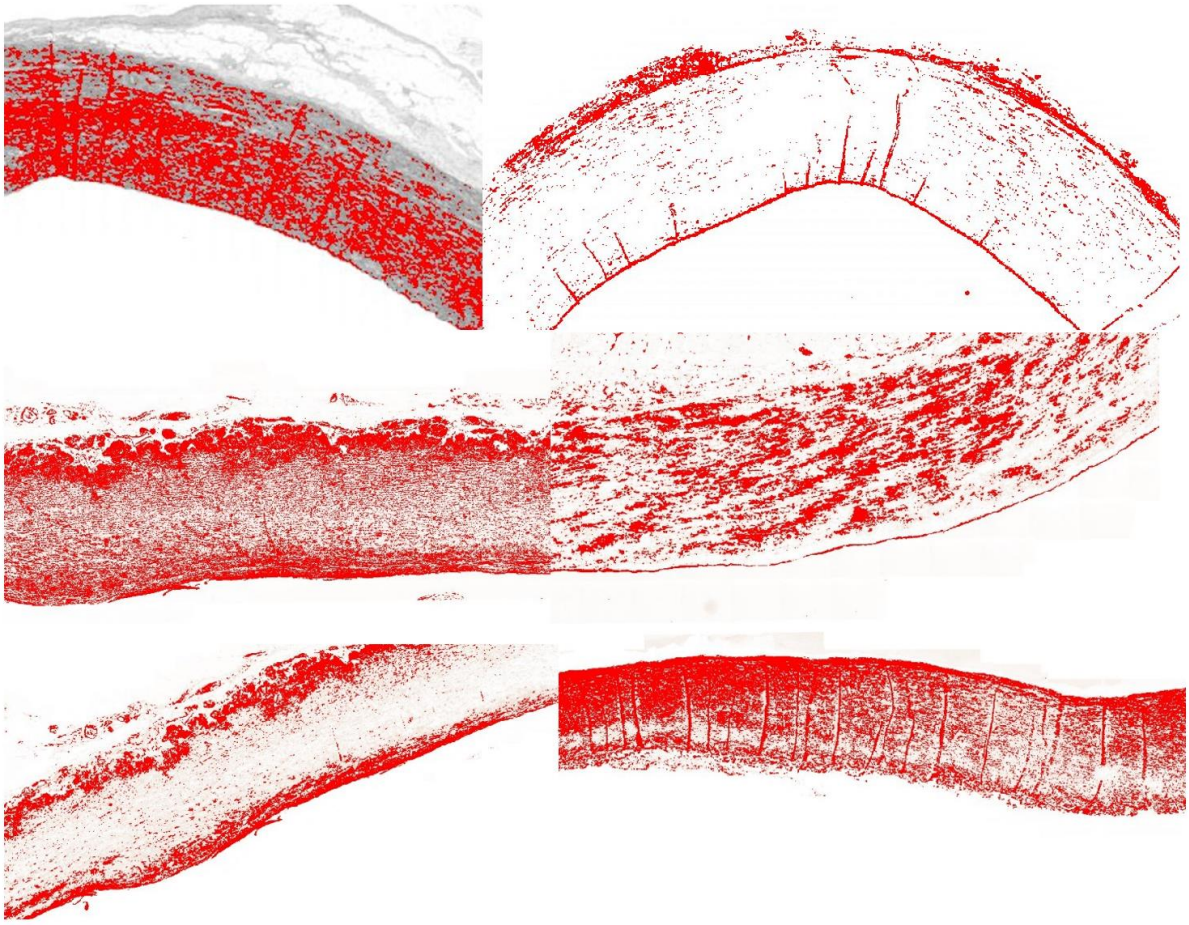

Figure 2.8: Colour deconvolution image of EVG stained specimen (Top left) and Massons stained specimen (Top right). Elastin and collagen deposition is marked in red. Aortic root clumping of collagen IV (Middle left and right). Aortic root aneurysm Collagen III distribution (Bottom left) and Collagen I distribution (Bottom right).

Collagen IV content did not show any regional variation in ascending aorta aneurysm patients (Table 2.23). Content showed great variation amongst root aneurysm samples and between root regions (Table 2.21) (Figure 2.8) p value = >0.99. Non-aneurysmal results are presented in Table 2.24.

Patient	Tissue samples	Distal (%)					Middle (%)				Proximal (%)			
		Anterior	Posterior		Inner	Outer	Anterior	Posterior	Inner	Outer	Anterior	Posterior	Inner	Outer
1(1)	Ascending aorta	6.4	13.3		23.1	6.4	7.5	9.0	10.4	12.5	2.4	7.8	1.9	2.0
1(2)	Ascending aorta	6.0	12.1		22.5	7.5	7.8	9.7	11.3	11.9	3.5	8.4	2.5	2.5
2(1)	Ascending aorta	4.9	21.0		30.5	4.5	9.5	9.0	11.4	12.8	16.7	13.3	12.5	35.5

2(2)	Ascending aorta	4.6	18.3	27.5	4.5	7.4	9.6	10.4	11.5	15.5	12.6	11.6	35.3
3(1)	Ascending aorta	8.8	14.8	1.5	8.7	7.8	13.8	6.7	5.3	3.7	4.0	5.1	4.1
3(2)	Ascending aorta	7.4	13.4	2.5	9.4	7.7	11.4	5.3	6.5	4.5	4.5	6.3	5.4
4(1)	Ascending aorta	5.3	13.5	25.5	8.9	6.6	2.4	17.8	5.2	7.1	9.0	3.0	4.8
4(2)	Ascending aorta	6.2	12.6	23.0	7.5	6.6	3.2	15.3	7.3	8.4	9.4	4.4	5.4
5(1)	Ascending aorta	8.6	6.5	2.1	1.0	6.3	11.6	3.7	6.3	4.9	10.1	7.4	7.0
5(2)	Ascending aorta	7.4	5.7	3.5	1.5	7.6	10.5	4.7	7.2	5.7	10.6	7.4	6.9
6(1)	Ascending aorta	10.1	14.9	8.9	7.3	22.0	14.3	19.4	13.6	7.4	3.3	7.3	4.4
6(2)	Ascending aorta	10.4	13.3	9.0	6.0	20.1	13.2	18.5	12.5	8.8	4.5	8.2	5.2
7(1)	Ascending aorta	7.2	9.6	21.8	14.8	10.5	9.7	17.9	25.5	8.5	8.5	10.5	6.3
7(2)	Ascending aorta	7.5	9.3	20.4	13.3	10.3	9.6	14.2	24.3	8.2	7.2	11.0	7.3
Average		**7.2**	**12.7**	**15.9**	**7.2**	**9.8**	**9.8**	**11.9**	**11.6**	**7.5**	**8.1**	**7.1**	**9.4**

Table 2.23: Collagen IV analysis via colour deconvolution in aneurysmal patients.

Patient	**Tissue samples**	**Distal (%)**				**Middle (%)**				**Proximal (%)**				**Root (%)**
		Anterior	**Posterior**	**Inner**	**Outer**	**Anterior**	**Posterior**	**Inner**	**Outer**	**Anterior**	**Posterior**	**Inner**	**Outer**	**Root**
1(1)	Ascending aorta	5.3	6.0	7.3	11.1	19.7	18.7	16.9	25.8	25.5	20.5	17.9	22.8	20.5
1(2)	Ascending aorta	5.2	7.2	8.2	11.5	20.6	18.7	16.3	24.0	24.3	19.9	18.9	22.0	21.5
2(1)	Ascending aorta	11.0	6.5	24.9	15.4	16.6	21.6	21.6	27.4	12.3	23.6	20.7	22.6	22.5
2(2)	Ascending aorta	11.0	8.8	23.0	15.1	17.4	21.5	19.4	29.4	11.5	22.2	21.3	22.5	23.5
3(1)	Ascending aorta	13.6	13.8	22.2	12.8	17.5	27.7	19.4	29.4	23.0	29.9	25.5	20.4	20.5
3(2)	Ascending aorta	14.2	13.5	23.2	13.8	18.3	28.5	19.2	31.4	22.3	30.8	24.0	21.0	22.6
4(1)	Ascending aorta	19.3	28.2	17.6	18.3	19.6	27.6	7.1	16.0	21.8	15.7	28.2	24.8	18.5
4(2)	Ascending aorta	20.7	25.9	15.4	19.5	21.3	26.6	8.5	16.4	21.5	16.2	28.4	23.6	19.5
5(1)	Ascending aorta	23.3	26.8	34.2	36.6	22.1	37.4	17.5	29.6	30.9	23.5	15.3	23.9	25.2
5(2)	Ascending aorta	23.7	25.9	33.2	35.5	22.3	36.5	16.4	29.3	31.3	22.4	14.7	23.6	21.0
Average		**14.7**	**16.3**	**20.9**	**19.0**	**19.5**	**26.5**	**16.2**	**25.9**	**22.5**	**22.5**	**21.5**	**22.7**	**21.5**

Table 2.24: Collagen IV analysis via colour deconvolution in non-aneurysmal patients.

2.7 Discussion

This study has demonstrated that aneurysmal and non-aneurysmal aortas display a quantifiable difference in collagen content, with aneurysmal aortas demonstrating a significantly higher collagen content. Furthermore, it was shown that the aneurysmal aortic root sinuses had the highest overall collagen content, followed by the aneurysmal proximal ascending aorta.

Identified imitations included variation in analysis, small number of aortic root patients, reproducible tissue excision from aneurysmal patients, and use of cadavers for normal aortas.

Most previous histological aneurysm studies have focused on BAV aneurysms [28,36], and dissecting abdominal aneurysms, showing incremental increases in collagen content [32,37,38,39], with broken collagen crosslinks and impaired synthesis [40,41]. Some have reported no change [32,42]. The increases in collagen deposition and altered collagen synthesis is supported in our findings. Core protein composition in the aneurysmal ascending aorta showed that collagen was extensively distributed, and greater in qualitative and quantitative measurements.

Elastin fibre fragmentation was moderate and extensive in aneurysmal samples, with a significantly reduced overall quantity compared to non-aneurysmal samples, supporting studies suggesting a 50% decrease in diseased samples [32]. Elastic fibre fragmentation and loss [24,40,43,44,45], and decreased elastin content [38,39,46] are frequently reported. The aneurysmal ascending aorta has been shown to have reduced elastic properties, and is thought to be associated with greater compliance under stress [28,36]. These functional characteristics are supported by our findings of generalised reduced elastin content throughout pathological samples.

The normal aortic root has many complex and variable protein components. Interleaflet triangles contain primarily collagen fibres [26], whereas the sinuses are primarily elastic lamellae [26]. The pathological aortic root demonstrates increased elastin fibre fragmentation, and reduced elastin fibre content, as well as decreases in collagen I and III subtypes. There have been variable reports on the effects of collagen content. Weakness of the aortic wall and aneurysmal dilatation has been associated with increased collagen content but decreased density and concentration [37,38,39] in affected areas. However, in contrast to these findings, weakness of the aortic wall has also been associated with a decrease in collagen content [39]. Detailed studies on the ascending aorta and aortic root aneurysm histopathology (including comparisons) are scarce and therefore comparisons are difficult to make.

Collagen subtypes in the ascending aorta comprise collagen type I, III and IV [26,39], whereas the aortic root consists of fibrous regions, arterial tissue within the SOV [47] and is without elastic

lamellae [41,44,48,49,50]. Collagen I, III, and IV have been reported in thick bundles and in increased amounts compared to controls [18,32,42,51], with collagen IV shown to be reduced or missing in other aneurysms [18]. The ratio of collagen I and III has been reported as important and reductions in type III collagen have been reported in familial aneurysmal groups [18]. The greatest consistency has been in reporting increases in collagen I and III in media and adventitia of aneurysmal walls [52]. There is great variability in collagen subtypes in aneurysmal and dissection study results with most reporting higher amounts of type I, III and IV in pathology. We report collagen I as having the greatest variability between the root and ascending aorta, but there is no evidence to compare, identifying a significant gap in current knowledge.

Regional analysis found no difference between inner, outer curvature, anterior or posterior regions in the ascending aorta in degree of elastin loss and collagen content [31,33] but numerous studies reported lateral wall changes [32,34]. Regional analysis of the root and ascending aorta identified extremes of collagen and elastin in the proximal inner regions, outer regions, and the aortic root itself, suggesting pathological changes occur in these regions more frequently. Comparisons on regional analysis of the aorta are scarce, identifying again a significant gap in current knowledge.

2.8 Conclusion

We have identified clear microstructural differences between the ascending aorta and aortic root in elastin, collagen, and collagen subtypes. The aneurysmal aortic root appears to show an increased collagen deposition and fibrosis and reduced elastin content in valvular and vascular regions compared to the ascending aorta.

These findings suggest a susceptibility to progressive pathology in the aortic root. Consideration should be given to identification of the aortic root as a structurally unique region of the aortic complex. Consequently, aortic root aneurysms should be considered a unique pathological entity, distinct from aneurysm in the remainder of the aortic complex. The authors recognise that, to obtain a greater understanding of the unique nature of the aortic root, larger cohort studies of the aortic root structure in cases of isolated aortic root aneurysms are required.

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CHAPTER THREE

The functional limits of the aneurysmal aortic root. A unique pressure testing apparatus.

Published in the Journal of Cardiothoracic Surgery, September 2020

Chapter 2 identified clear microscopic differences between the aortic root and ascending aorta in aneurysms and this stimulated investigation into the how this may affect the macroscopic structure of the aorta.

Firstly, this would involve an ex-vivo rupture testing of pig aortic root and ascending aortas. My role involved design of the testing apparatus, construction of the apparatus, and laboratory rupture testing, as well as analysis of the results.

Experimental results that suggest structural differences between the aortic root and ascending aorta would lead onto in vivo pig rupture testing (Chapter 4) to identify if patterns of variation exist and if structural abnormalities influence aortic integrity.

3.1 Statement of authorship

Statement of Authorship

Title of Paper	The functional limits of the aneurysmal aortic root. A unique pressure testing apparatus
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Contribution to the Paper	Primary investigator and author Methodology Experimentation Publication formulation		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	18/2/21

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Experimental planning Publication planning guidance Publication editing		
Signature		Date	19/2/21

3.2 Manuscript summary

Title

The functional limits of the aneurysmal aortic root. A unique pressure testing apparatus.

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3.3 Abstract

Background

The aortic root has unique embryological development and is a highly sophisticated and complex structure. In studies that report on the biomechanical characteristics of the thoracic aorta, distinction between the aortic root and ascending aorta regions is non-existent. Our objective is to determine the maximal pressures at which dissection or tissue failure occurs in both the aneurysmal aortic root and ascending aorta and to analyse any differences. This may help guide preoperative monitoring, diagnosis, and the decision for operative intervention for aortic root aneurysms in the normal and susceptible populations.

Methods

We developed a simple aortic root and ascending aorta pressure testing unit in series. Ten fresh pig hearts were obtained from the local abattoir (n = 5 aortic root and n = 5 ascending aorta for comparison). Using a saline filled needle and syringe, artificial fluid-filled aneurysms were created between the intima and medial layers of the aortic root. The aorta lumen was then progressively filled with saline solution. Pressure measurement was taken at time of loss of tissue integrity, obvious tissue dissection or aneurysm rupture, and the tissue structure was then visually examined.

Results

In the aortic root, mean maximal pressure (mmHg) at tissue failure was 208 mmHg. Macroscopic examination revealed luminal tears around the coronary ostia in 2/5 specimens, and in all specimens, there was propagation of the dissection in the aortic root in a circumferential direction.

In all ascending aorta specimens, the maximal aortic pressures exceeded 300 mmHg without tissue failure or dissection, and eventual apparatus failure.

Conclusions

Our results indicate that the aneurysmal aortic root is at greater risk of rupture and dissection propagation at a lower luminal pressure than the aneurysmal ascending aorta. With further analysis, this could guide clinical and surgical management.

3.4 Background

Ascending aortic dissection is the most common catastrophe of the aorta; and two to three times more common than that of the abdominal aorta [1]. Mortality rate of untreated acute dissection involving the ascending aorta is about 1–2% per hour during the first 48 hours, and the first documented case was King George II in 1760 [2]. Constant exposure to high pulsatile pressure and shear stress leads to a weakening of the aortic wall in susceptible patients resulting in an intimal tear [3]. Most of these tears take place in the ascending aorta, usually in the right lateral wall where the greatest shear force on the aorta occurs [4].

Aneurysms of the aortic root arise relatively deep within the heart and because of frequently associated complications, such as aortic insufficiency, present a more complicated problem than the more distal aneurysms of the ascending aorta [5]. The aortic root has unique embryological development and is a highly sophisticated and complex structure. Its optimal structure ensures dynamic behaviour in flow characteristics, coronary perfusion and left ventricular function. In studies that report on the biomechanical characteristics of the thoracic aorta, distinction between the aortic root and ascending aorta regions is non-existent. Aortic root replacement is associated with high mortality and morbidity and is therefore frequently avoided in cases of acute aortic dissection for fear of increased surgical risk. Approximation of the aortic wall layers within the dissected sinuses of Valsalva with a biological glue and subsequent supracoronary aortic replacement offers a simple and efficient method of preserving the native valve and abolishing the aortic insufficiency when it is caused by the distortion of root anatomy. However, non-curative root repair can result in late development of several pathologies, which, especially after use of glue, necessitate challenging redo surgeries [6].

The initial decision regarding the management of the aortic root in type A aortic dissection (TAAD) is whether to repair or replace the dissected sinus segments [7]. The standard indications for aortic root replacement (ARR) in the setting TAAD are extensive tissue destruction, the presence of a concomitant aortic root aneurysm ≥4.5 cm, or a known connective tissue disorder. The most common pathology observed is a primary intimal tear located in the ascending aorta with extension of the dissection flap into the noncoronary cusp, and relative preservation of the left and right

coronary sinuses. Rarely are the aortic valve cusps or annulus impacted by the dissection process [7].

A meta-analysis of aortic valve-preserving surgery in TAAD containing 2402 patients from 19 observational studies revealed that, in 95% of the patients, the surgery consisted of conservative root management and supracoronary aortic replacement, while only 5% underwent a curative root repair by valve-sparing root replacement (VSRR) (reimplantation or remodelling). In a large aortic dissection repair centre, 10% of the patients with aortic root dissection, a non-curative root repair using tissue glue was performed at the surgeon's discretion [6].

Coady and colleagues studied 370 patients with thoracic aneurysms (201 ascending aortic aneurysms), during a mean follow-up of 29.4 months, the incidence of acute dissection or rupture was 8.8% for aneurysms less than 4 cm, 9.5% for aneurysms of 4 to 4.9 cm, 17.8% for 5 to 5.9 cm, and 27.9% for those greater than 6 cm. In this study, the median size of the ascending aortic aneurysm at the time of dissection or rupture was 59 mm. The growth rate ranged from 0.08 cm/year. for small (4 cm) aneurysms to 0.16 cm/year. for large (8 cm) aneurysms [8].

The risk of aortic dissection and rupture is often related to the transverse diameter of the aortic sinuses. It is rare with diameters less than 50 mm except in cases of family history of dissection or inpatients with LDS. Surgery is usually recommended when the diameter of the aortic root reaches 50 mm. Patients with family history of aortic dissection, or the diagnosis of LDS should be operated on when the transverse diameter exceeds 40 mm [8].

Our objective is to determine the maximal pressures at which dissection or tissue failure occurs in the aortic root compared to that of the ascending aorta, and determine the pattern of propagation of pseudoaneurysm within the aortic root at these pressures. This may help guide preoperative monitoring, diagnosis, and the decision for operative intervention for aortic root aneurysms in the normal and susceptible populations.

3.5 Methods

We developed a simple aortic root and ascending aorta pressure testing unit in series (Fig. 3.1). This apparatus consisted of an aortic root and ascending aorta pig specimen, a pressure transducer measuring in mmHg (National instruments Pty Ltd., Austin, TX), two large vessel clamps, and a 50 ml syringe filled with saline solution with a 21-gauge needle.

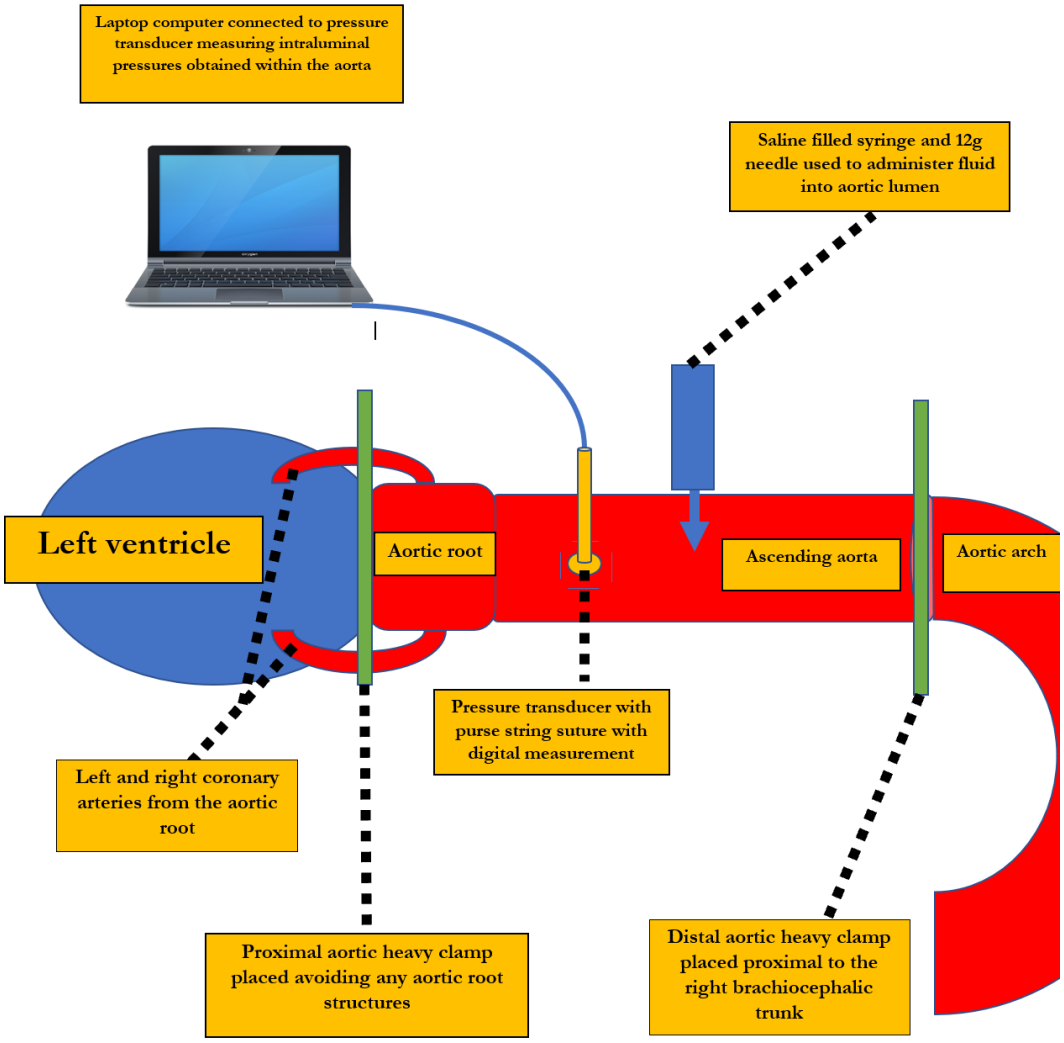

Figure 3.1: Diagram of the aortic root and ascending aorta pressure apparatus. This diagram is labelled with the main features of the apparatus. Two clamps are placed proximal and distal to isolate the aortic root and ascending aorta. The administration of saline into the lumen of the aorta and the pressure transducer connected to a nearby laptop to measure and record the maximal pressures before aortic or apparatus failure is demonstrated.

Pig hearts (n = 5) were obtained fresh from local abattoirs which included the heart and ascending aorta attached to the brachiocephalic trunk on the right side. In addition, pig hearts (n = 5) were obtained for testing on the ascending aorta alone (excluding the aortic root). Animal ethics approval was not required according to local South Australian Health and Medical Research Institute (SAHMRI) and Preclinical, Imaging, and Research Laboratories (PIRL) protocols.

The aorta was dissected proximally to the left ventricle to include the entire aortic root. The dissection then extended distally to the distal ascending aorta. The proximal limits were the left ventricle and distal limits was the brachiocephalic trunk.

Large vessel clamps were applied to the proximal and distal limits of the aorta (Figs. 3.2 and 3.3). The most distal region was limited by the branches of the aortic arch. The most proximal region limited by the left ventricle and careful avoidance of the aortic root structures and left and right coronary arteries. Using a size 11 scalpel blade, a small incision was made in the proximal ascending aorta distal to the aortic root, and the pressure transducer inserted within the ascending aorta lumen. A purse string suture was placed circumferentially around the incision to prevent dislodgement of the transducer during pressurisation. The pressure transducer was connected to a laptop computer and pressure measurements taken in real time using LabVIEW (National Instruments Pty Ltd., Austin TX). Saline solution was aspirated into a 50 ml syringe and 21-gauge needle applied. The needle was then inserted between the intimal and medial layers at the level of the coronary ostia to create an aneurysm in the aortic root testing and in the region of the proximal aorta during the ascending aorta testing. Saline solution was administered until a visible aneurysm was created identifying disruption to the tissue layers. Using this same syringe and needle, saline solution was administered into the lumen of the ascending aorta between to distal and proximal clamps until the lumen was filled and pressurised. Concurrent pressure measurements (mmHg) were taken and recorded during filling (Fig. 3.4). Pressure measurements was taken at time of loss of tissue integrity, obvious tissue dissection or aneurysm rupture. The pressure measurement was determined to be the maximal pressure at time of loss of aortic root tissue integrity. The aortic root and ascending aorta were then opened, and the tissue microstructure was examined.

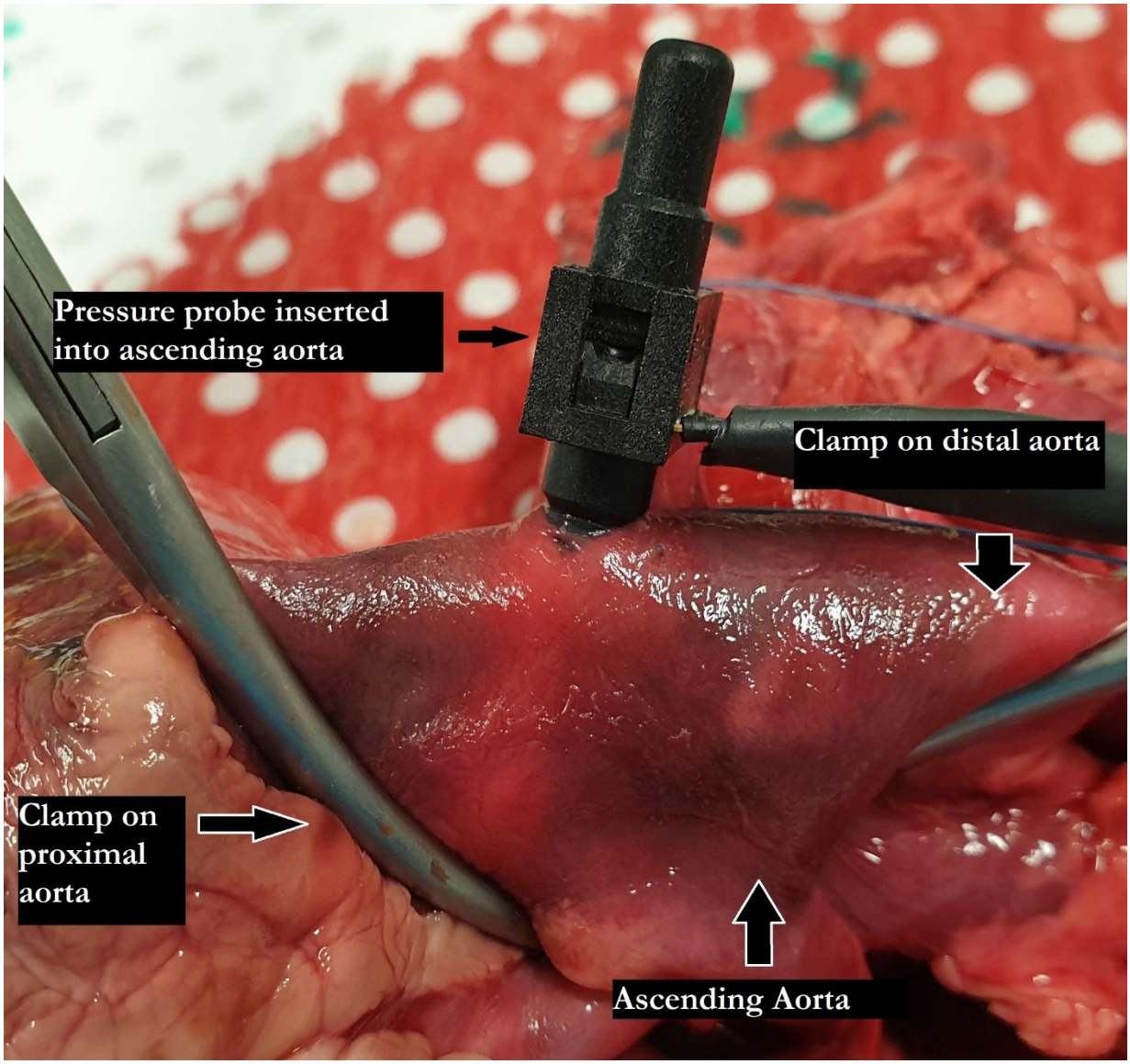

Figure 3.2: Aortic root and ascending aorta apparatus photograph. The proximal clamp is sitting at the most proximal portion of the aortic root clear of any aortic root structures. The pressure probe sits at the start of the proximal ascending aorta and distal clamp at the distal ascending aorta. Purse string sutures are yet to be placed around the pressure probe.

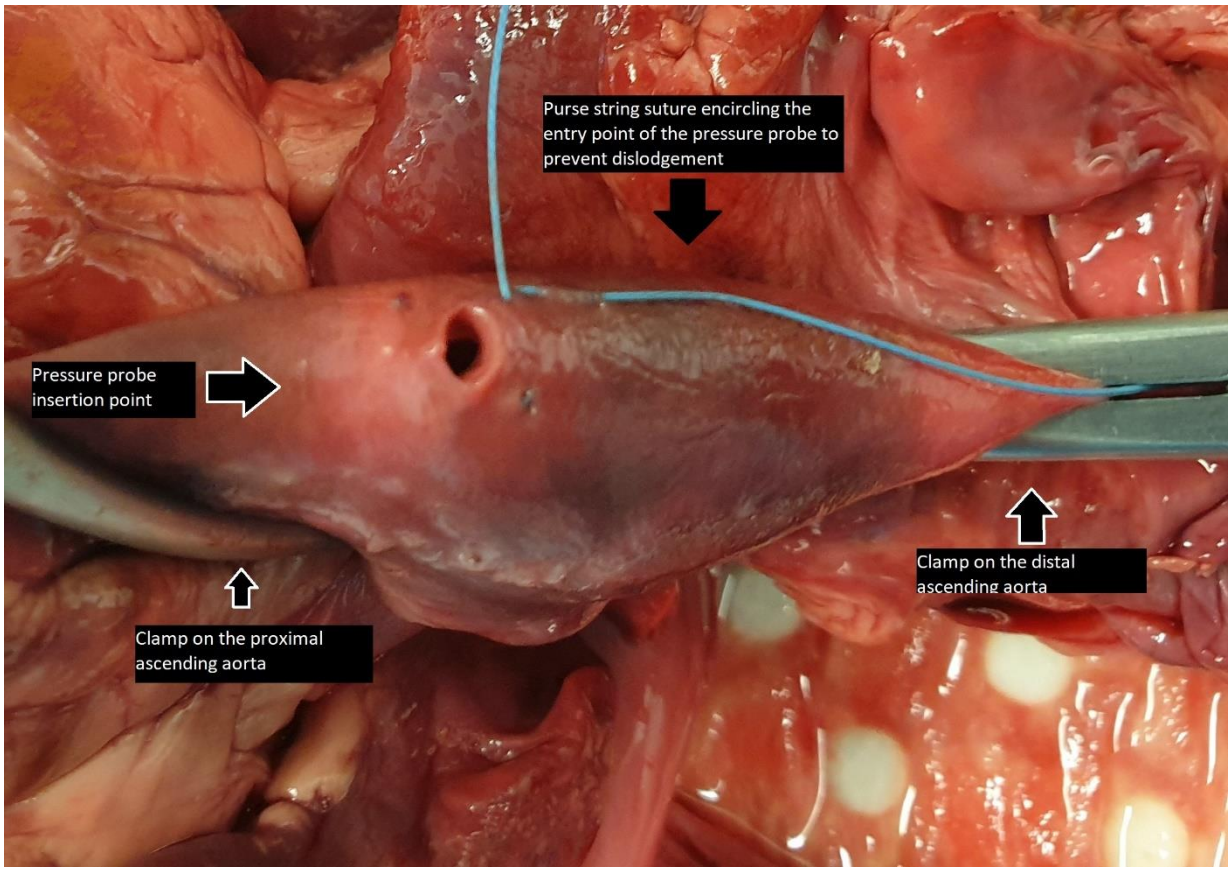

Figure 3.3: Overhead view of the aortic root and ascending aorta apparatus. The proximal clamp and distal clamp are at the proximal and distal limits of the thoracic aorta. The purse string suture is placed around the site of the pressure probe in the proximal ascending aorta.

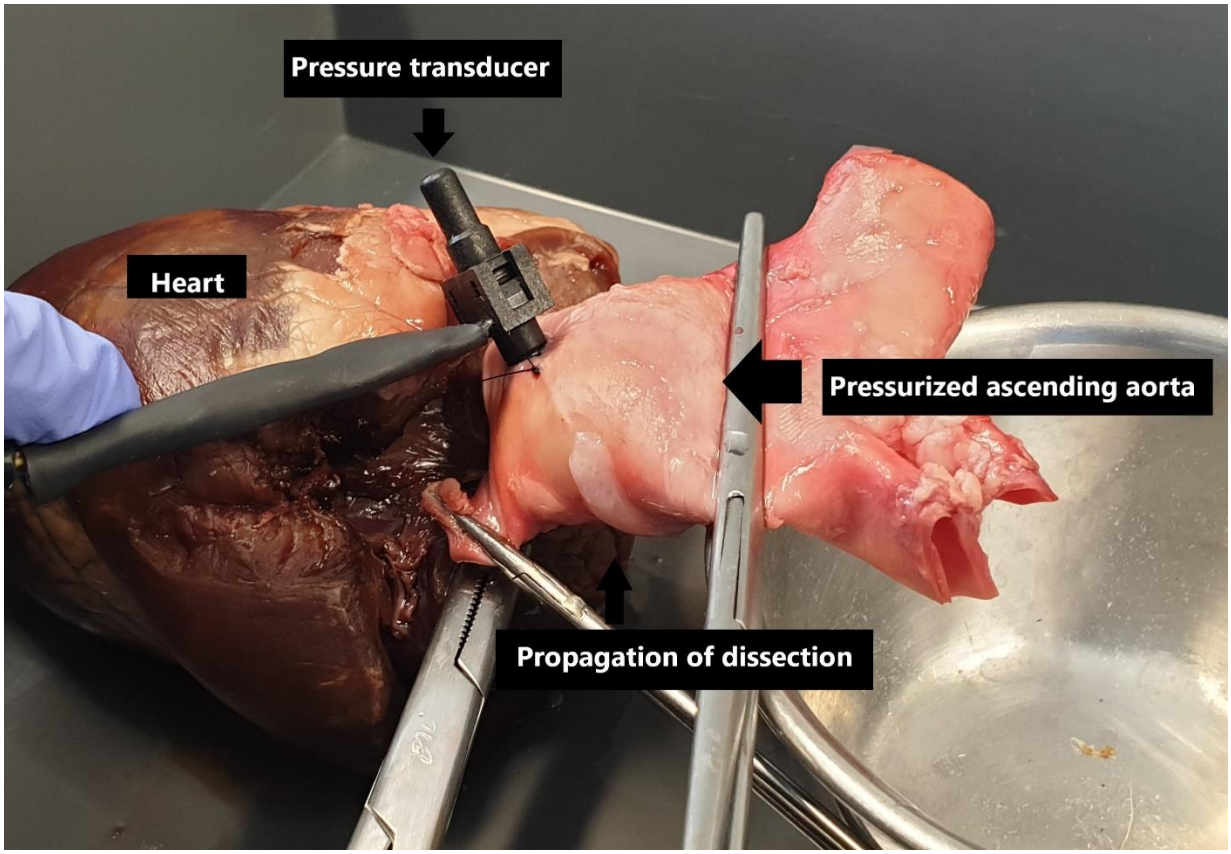

Figure 3.4: Photograph showing the aortic root and ascending aorta apparatus during pressure testing. The proximal clamp is positioned proximal to the aortic root, with small clamps placed on the left and right coronary arteries to prevent fluid leak. The pressure probe with associated purse string suture is positioned in the proximal ascending aorta, distal to the coronary arteries.

3.6 Results

Pressure measurements were conducted on 5 pig aortic root specimens, and maximal pressure determined at the time of loss of tissue integrity. The mean maximal pressure (mmHg) at tissue failure was 208 mmHg (Table 3.1). Macroscopic examination revealed luminal tears around the coronary ostia in 2/5 specimens (Figs. 3.5 and 3.6), and in all specimens, there was propagation of the pseudoaneurysm dissection in the aortic root in a circumferential direction.

Pig specimen aortic root	Maximal pressure (mmHg)	Macroscopic characteristics	Pig specimen ascending aorta only	Maximal pressure (mmHg)	Macroscopic characteristics
1	180	• Tissue dissection at site of pressure transducer • Circumferential spread of dissection	**1**	300+	• No loss of tissue integrity • Apparatus failure
2	200	• Tissue dissection at site of pressure transducer • Luminal tear at coronary ostia • Circumferential spread of dissection	**2**	300+	• No loss of tissue integrity • Apparatus failure
3	220	• Tissue dissection at site of pressure transducer • Luminal tear at coronary ostia • Circumferential spread of dissection	**3**	300+	• No loss of tissue integrity • Apparatus failure
4	200	• Tissue dissection at site of pressure transducer • Circumferential spread of dissection	**4**	300+	• No loss of tissue integrity • Apparatus failure
5	240	• Tissue dissection at site of pressure transducer • Circumferential spread of dissection	**5**	300+	• No loss of tissue integrity • Apparatus failure

Table 3.1: Pig pressure measurements of the aortic root and ascending aorta.

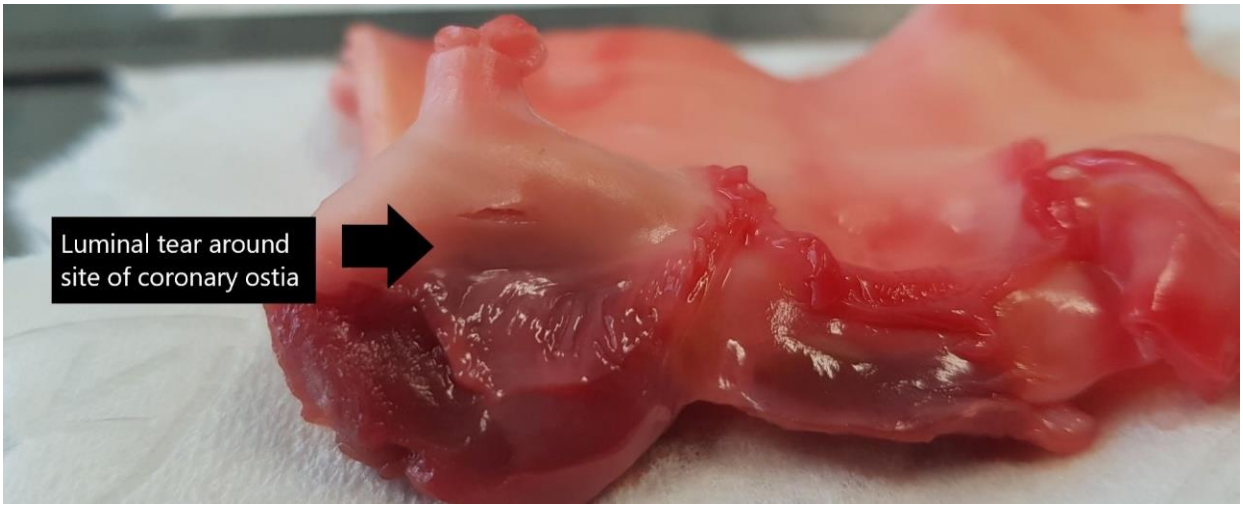

Figure 3.5: Photographs of the aortic root region cut open to examine the internal structures. What both photographs show are small tears in the lumen in the coronary ostia and sinus tissue regions as shown by the black arrow. The remaining valvular apparatus remained intact.

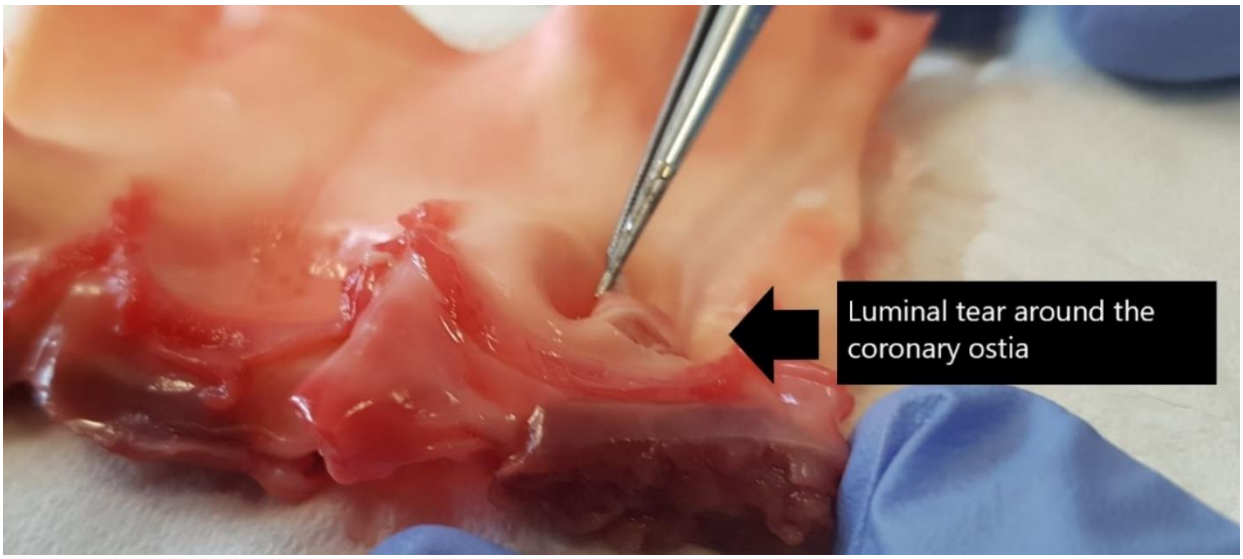

Figure 3.6: Photographs of the aortic root region cut open to examine the internal structures. What both photographs show are small tears in the lumen in the coronary ostia and sinus tissue regions as shown by the black arrow. The remaining valvular apparatus remained intact.

Pressure measurements were conducted on 5 pig ascending aorta specimens (excluding the aortic root), and maximal pressures recorded at the time of loss of tissue integrity or apparatus failure (Fig. 3.7). The median maximal pressure post rupture was 200 mmHg (range 180 to 240), compared to greater than 300 mmHg pre rupture for all specimens. This was significantly different. In all specimens, the maximal aortic pressures exceeded 300 mmHg without tissue failure or dissection,

and eventual apparatus failure (Table 3.1). Macroscopic examination revealed no luminal tissue dissection or tearing. There was no evidence of aneurysms dissection (Fig. 3.8).

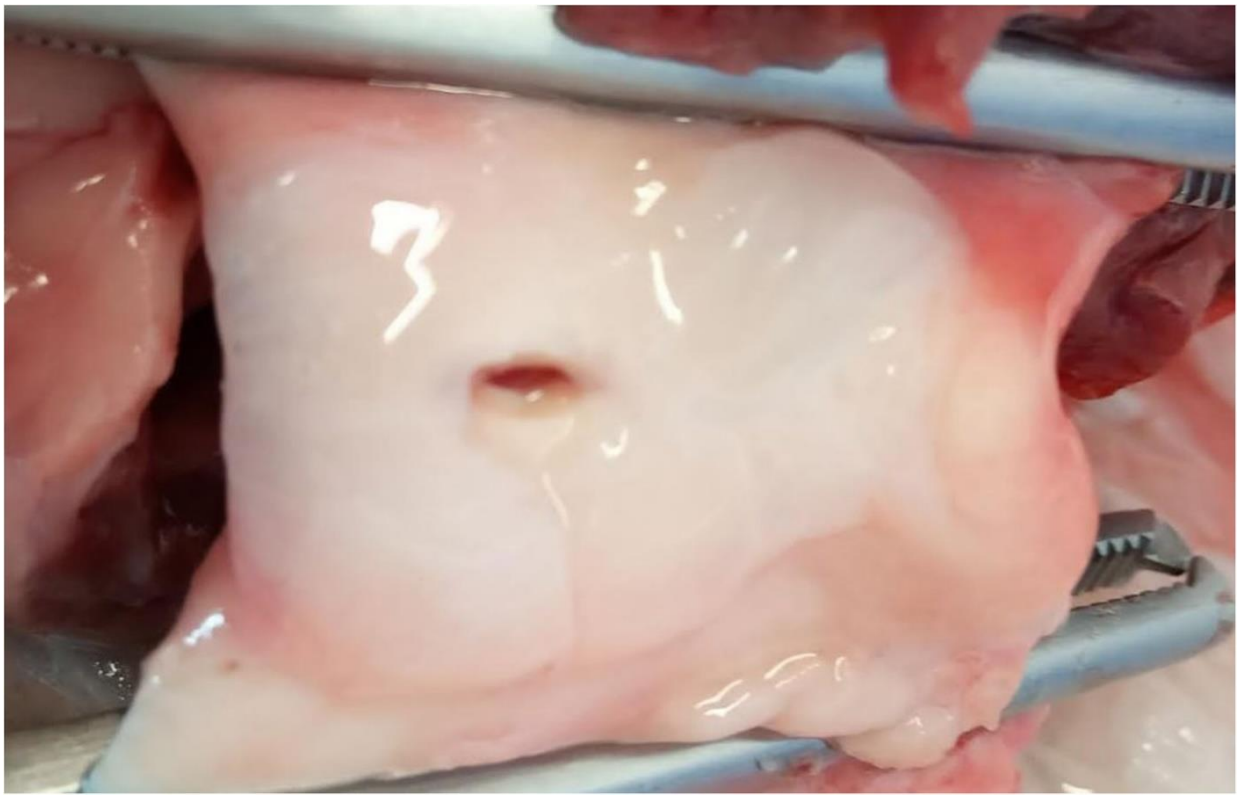

Figure 3.7: Photograph of the aortic root region taken from the superior aspect. The spreading of the injected saline into the aortic layers and propagating as a dissection in a circumferential pattern is seen. The superior clamp is proximal, and the inferior clamp is distal. The pressure probe is removed from the centre of the image for clarity.

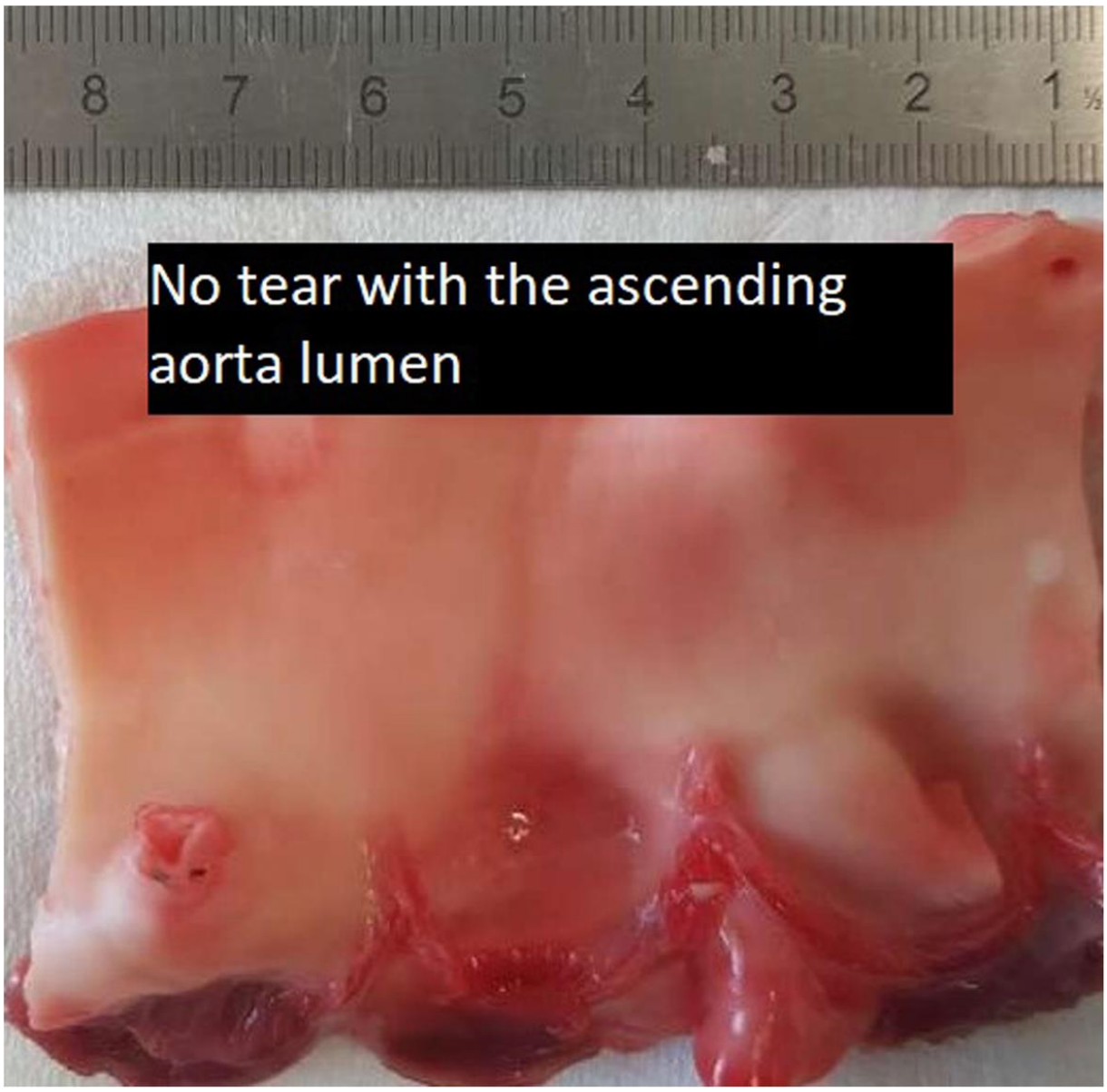

Figure 3.8: Photograph of the internal structures of the aortic root and ascending aorta following pressure testing. The photographs show an intact ascending aorta lumen with no tearing of the ascending aortic tissue in this test sample.

3.7 Discussion

The aortic root is a unique embryological, anatomical, and physiological structure within the aortic complex. Consequently, diagnosis and surgical management of aortic root aneurysms need to be tailored accordingly. Diagnosis and subsequent management are determined by aneurysm size, rate of progression, and predisposing factors such as valvular pathology and genetic conditions such as MFS and LDS. There are no reported studies of the macroscopic integrity of the aneurysmal aortic root specifically, or its propensity to rupture at certain aortic pressures. All studies to date have

looked at sectioned specimens and none have examined the effects of various biomechanical stresses on the complete intact aortic apparatus. [9,10,11,12,13,14,15].

It has been reported that a significant proportion of dissection patients do not seem to have aneurysmal aortas at the time of presentation. On review of the International Registry of Aortic Dissections (IRAD) data, of 591 patients reviewed, almost 60% had diameters < 5.5 cm and 40% had aortic diameters < 5 cm. Suggestions have been made for utilisation of genetic markers, biomarkers, and functional studies to better predict susceptible patients to aortic dissection. If the aortic root represents a unique structure with a predisposition to rupture compared to the ascending aorta, then do we need even more aggressive monitoring, management, and consideration for intervention in aneurysmal proximal ascending aorta and aortic root pathology?

We have looked at 10 pig specimens comparing the maximal aortic luminal pressures and aneurysmal dissection patterns within the ascending aorta and aortic root using a unique pressure testing apparatus. There are several limitations to our study. First, the use of a clamp at the most proximal part of the aortic root and a clamp in the most distal part of the ascending aorta may cause distortion to the aortic root and affect the pressures recorded. All attempts were made to place the proximal clamp devoid of any aortic root tissue in all experiments. Despite this limitation, clamping allowed for localisation of the maximal pressure to a smaller area and precise administration of luminal fluid. Additionally, the ascending aorta pressure monitor required insertion into the proximal ascending aorta lumen itself causing disruption of the associated tissue structure. Although no major disruption of the tissue occurred at this site under high pressures, this may have been an area of weakness and minor fluid leak resulting in some skewing of obtained results.

Second, due to fresh pig abattoir animal preparation prior to testing, significant mechanical injury was seen in the cardiac muscle and subsequently not amenable to use in the testing process. This required placement of the proximal clamp to prevent leaking of the intraluminal fluid through the cardiac internal and external tears.

Third, this static pressure model may not reflect the beating heart velocity of ventricular contraction (dp/dT) changes that occur in a clinical setting, but more reflects a measure of the pressure differences and tissue changes that occur under high luminal pressures in different parts of the thoracic aorta.

Simulation models have focused on a few areas around the thoracic aorta including valvular function, aortic aneurysms, and aortic dissections [10,16,17,18,19,20,21,22,23,24]. The studies listed have reported on the flow characteristics around the aortic valve particularly in patients with BAV and its effects on haemodynamics. Other simulations have focused on reproduction of the aortic aneurysm and dissection process using 3-dimensional (3D) aortic models derived from computer tomography (CT) scanning. Zannoli and colleagues in 2002, 2004, and most recently in 2007 [23]

created a mechanical simulator to mock the cardiovascular system reproducing the frank-starling mechanism. Using a balloon and adjustable external reservoir with the aorta simulated by a rubber tubing, they aimed to create a device to reduce the high mortality in the presurgical phase of aortic dissections. They did this by three main mechanisms, improving coronary perfusion, slowing the dissection process, and recovering some of the mechanical efficiency of the cardiac-arterial junction [23]. The disadvantage of such approaches is the associated complexity and resources required to produce these models as well as the lack of gold standard validation in several cases.

Our results indicate that the aneurysmal aortic root tissues are at greater risk of rupture and dissection propagation at lower aortic pressure than the ascending aorta. Future testing of aortic root and ascending aorta pressure limits should include the incorporation of a dynamic pressure model using dp/dt and frank starling forces to replicate the cardiac cycle as accurately as possible. Further testing of greater tissue numbers is needed to confirm these findings, but consideration should be for much closer monitoring of aortic root aneurysms, strict blood pressure control of patients with known aortic root aneurysms and earlier intervention of aortic root aneurysms.

A limitation of this method of creating an aneurysm does not completely mirror the normal, chronic changes of aortic aneurysm formation including the thinning of the tissues, weakening of the connective tissues, and local stress points related to atherosclerosis (penetrating aortic ulcers) which could contribute to the development of aortic dissection.

3.8 Conclusions

The aortic root is a unique embryological, anatomical, and physiological structure that demonstrates a specific pattern of aneurysmal pathology when compared to the ascending aorta. No studies to date have tested the limitations of the weakened aortic root tissue, and we have reported on a reliable and reproducible aortic pressure model to identify the differences between these two structures. Knowledge in the pressure and structural limitations of the aneurysmal aortic root could guide clinical management of patients with known aneurysms, monitoring of progression and growth of aneurysms and ultimately surgical repair and replacement.

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CHAPTER FOUR

The susceptibility of the aortic root. Porcine aortic rupture testing under cardiopulmonary bypass

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This chapter has a 2-fold experimental strategy. Given the earlier experimental findings in Chapter 2 and Chapter 3 which identify a clear difference between the microscopic and macroscopic structure of the ascending aorta and aortic root in health and disease, there is a need to test the limits of these structures under conditions that more closely replicate the physiological flows and pressures that the body produces.

Secondly, the literature reports scarce information on prevalence of isolated root dissection/rupture, yet identifies the absolute importance of this structure in the overall survival following dissection repair. There is a need therefore to test under maximal loading conditions, which part of the root is most susceptible to failure and the simultaneous impact of the ascending aorta.

4.1 Statement of authorship

Statement of Authorship

Title of Paper	The simulation of thoracic ascending aorta and aortic root rupture and pathological analysis: Realtime porcine modelling under cardiopulmonary bypass
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Principal Author

Name of Principal Author (Candidate)	Timothy Luke Surman		
Contribution to the Paper	Primary investigator and author Specimen preparation Histological analysis Surgical experimentation Publication formulation		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	13/2/21

Co-Author Contributions

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ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Name of Co-Author	Chris Christou		
Contribution to the Paper	Animal ethics and governance guidance Animal experimental design planning Animal experimental process Publication planning guidance Publication editing		
Signature		Date	17/02/2021

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Statement of Authorship

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Overall percentage (%)	60%		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	John Abrahams		
Contribution to the Paper	Experimental concept and design Publication planning guidance Publication editing		
Signature		Date	13/2/2021

4.2 Manuscript summary

Title

The susceptibility of the aortic root: porcine aortic rupture testing under cardiopulmonary bypass

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4.3 Abstract

Background

In our earlier study on the functional limits of the aneurysmal aortic root we determined the pig root is susceptible to failure at high aortic pressures levels. We established a pig rupture model using cardiopulmonary bypass to determine the most susceptible region of the aortic root under the highest pressures achievable using continuous flow, and what changes occur in these regions on a macroscopic and histological level. This information may help guide clinical management of aortic root and ascending aorta pathology.

Methods

Five pigs underwent 4D flow MRI imaging pre surgery to determine vasopressor induced wall sheer stress and flow parameters. All pigs were then placed on cardiopulmonary bypass (CPB) via median sternotomy, and maximal aortic root and ascending aorta flows were initiated until rupture or failure, to determine the most susceptible region of the aorta. The heart was explanted and analysed histologically to determine if histological changes mirror the macroscopic observations.

Results

The magnetic resonance imaging (MRI) aortic flow and wall sheer stress (WSS) increased significantly in all regions of the aorta, and the median maximal pressures obtained during cardiopulmonary bypass was 497mmHg and median maximal flows was 3.96L/m. The area of failure in all experiments was the non-coronary cusp of the aortic valve. Collagen and elastin composition (%) was greatest in the proximal regions of the aorta. Collagen I and III showed greatest content in the inner aortic root and ascending aorta regions.

Conclusions

This unique pig model shows that the aortic root is most susceptible to failure at high continuous aortic pressures, supported histologically by different changes in collagen content and subtypes in the aortic root. With further analysis, this information could guide management of the aortic root in disease.

4.4 Background

In the realm of aortic root and ascending aorta aneurysm management, there is no clear evidence-based distinction in the propensity to dissection or rupture between these two areas under various physiological conditions. Several animal models have been produced that have aimed to reproduce normal physiology (Table 4.1) however, no animal model has been developed that reproduces the high aortic pressures seen in humans. This is essential for testing of biomechanical limits of the human aortic root and ascending aorta. Repetitive high continuous pressure and shear stress leads to a weakening of the aortic wall in susceptible patients resulting in an intimal tear [1], commonly in the lateral wall [2]. Biomechanical distinction between the aortic root and ascending aorta regions is scarce, yet clinical management of aortic root and ascending aorta pathology remains the same.

Our objective is to use a pig model to replicate the real time stresses placed on the aortic wall and aortic root apparatus under cardiopulmonary bypass and under the influence of vasopressor administration, to show the clinical and radiological effects of the aorta under stress, and determine the areas of greatest susceptibility to failure. We set out to identify the histological characteristics of the aortic root and ascending aorta following the application of acute stresses on the aortic wall, and to correlate these with macroscopic findings.

Author	Purpose	Methodology	Findings
Angelos et al. 1993 (4)	To determine organ blood flow changes in a swine model using CPB to achieve return of spontaneous circulation (ROSC)	Swine model of 10 pigs placed on CBP following VF cardiac arrest	Low flow cardiopulmonary bypass model produces reproducible high resuscitation rates and ROSC.
Bufalari et al. 2015 (5)	To determine the most effective practice of left pneumonectomy	Swine model of 11 pigs undergoing left pneumonectomy	The most straightforward procedure required careful dissection of the pulmonary ligament, pulmonary veins, pulmonary artery, and finally bronchus
Eckhouse et al. 2013 (6)	To establish a reproducible model of aortic dilatation reproducing what happens in Thoracic abdominal aneurysm's development	Descending TAA's were induced in 7 pigs using collagenase and crystalline and tissue analysed	Tissue demonstrates aortic dilatation, aortic medial degeneration, and alterations in MMP/TIMP abundance consistent with TAA formation
Kofidis et al. 2014 (7)	To determine the feasibility of transapical cardioscopic surgery in a pig model	Transapical access to the ventricle was obtained in 5 pigs with right mini thoracotomy for central cannulation and CPB	Transapical approach allowed for good exposure and adequate surgical field for mitral valve, and aortic valve access, and atrial ablation and intra-aortic procedures
Lundemeon et al. 2013 (8)	To determine the effects of pulsed and non-pulsed CPB on microvascular fluid exchange	A total of 16 pigs were randomized to pulsatile (n=8) or non-pulsatile (n=8) CPB	No significant differences in the fluid extravasation rates were present between pulsed and non-pulsed cardiopulmonary bypass perfusion
Mariscal et al. 2018 (9)	To describe a surgical technique for swine lung transplantation and postoperative management 3 days postoperatively	Involved development of a protocol based on donor surgery, recipient surgery and postoperative care and sacrifice	This survival model can be used by lung researchers to assess development of primary graft dysfunction (PGD) and to test therapeutic strategies targeting PGD
Mickelson et al. 1990 (10)	To develop an alternative to canine models in testing for cardiopulmonary bypass research	15 pigs were divided into three groups to determine the optimum conditions during CPB to avoid complications of fluid shifts, metabolic acidosis, and hemoglobinuria	Determined that optimum blood flow rate for cardiopulmonary bypass in swine is in the range of 175-200 ml/kg min. Hyperosmolar priming solution is beneficial for CPB in swine to reduce fluid shifts, metabolic acidosis, and hemoglobinuria
Nicols et al. 2001 (11)	To determine the effect of changing FiO2-concentration on SvO2 in a swine model on CPB	8 mixed-gender swine were placed on CPB with an experimental and control group measuring percentage change in blood flow and oxygen delivery	Results suggest that decreased blood flow adjusting for increased SvO2 associated with high PaO2 did not result in significant reduction in adequacy of perfusion markers for organs studied
Oizumi et al. 2017 (12)	Development of a swine model for anatomical thoracoscopic lung segmentectomy training	33 pigs were used over a period of 5 years to train operators on segmentectomy via a hybrid (8) or thoracoscopic (23) approach. 3 pigs were converted to thoracotomy due to haemorrhage	Live swine model was considered a good choice for training surgeons on how to perform a minimally invasive lung segmentectomy in humans
Thalmann et al. 2019 (13)	Evaluation of several hybrid approaches for pulmonary valve replacement in a swine model	13 pigs were used using 4 different thoracotomy methods for valve implantation, and 5 cases used median sternotomy	Achieved implantation of 12/13 stented valves of which 41% were in the optimal position and 16% had paravalvular leakage. Lower partial sternotomy provided the best deemed approach

Table 4.1: Pig models utilizing cardiopulmonary bypass.

4.5 Material and Methods

All investigators complied with the 2011 "Guide for the Care and Use of Laboratory Animals", and approval by the South Australian Health and Medical Research Institute Animal Ethics Committee (SAHMRI AEC).

4.5.1 Animal preparation

Following our pilot study indicating differences between the rupture potential of the aortic root and ascending aorta in pig aortas [3], 5 female adult pigs were obtained for animal testing. All pigs weighed between 50 and 60 kg and were in good health. All animals had external jugular vein and

carotid arterial monitoring placed 2 days prior to the testing. Pigs underwent induction using 3–5 ml intramuscular ketamine, maintenance using 2–3% isoflurane, with ongoing ventilation and flow rate of 3–4 L/min. Ongoing monitoring of mean arterial pressure (MAP), systolic and diastolic blood pressure, heart rate and end title CO2 (etCO2) occurred with all experiments with observations recorded every 15–20 min.

4.5.2 Preoperative MRI imaging

All pigs underwent baseline MRI imaging at normal blood pressure and heart rate haemodynamics. All pigs then received a bolus noradrenaline dose of 5–6 ml at 4 mg/4 mL until systolic blood pressure exceeded 200 mmHg. Each pig then underwent MRI at systolic pressures > 200 mmHg to measure WSS and flow parameters.

All MRI scans were performed using a 3-Tesla Siemens Magnetom Skyra (Siemens Healthcare, Erlangen, Germany) (Fig. 4.1). The subject was positioned in dorsal recumbency within a custom-made MRI compatible positioning device. The subject's condition during MRI was monitored using invasive blood pressure monitoring and an MRI-safe pulse oximeter. Siemens Works In Progress (WIP) sequence, 4D Phase Contrast Flow (WIP 785A) was employed to quantify time-resolved flow within the aorta through cartesian sampling in three dimensions. The MRI images were analysed using Circle Cardiovascular Imaging (CVI42) version 5.10.1 Inc, Calgary, Alberta, Canada (Table 2). Each pig study underwent data cropping to identify the area of interest which included the aortic root, ascending aorta, arch, and descending aorta. The selected area then underwent pre-processing, whereby a tissue mask is defined. Offset correction and phase anti-aliasing was applied if unwanted flow or noise was identified. The vessel was then segmented, by tracing a centreline from the aortic valve to the descending aorta of which measurement will be determined, and vessel diameter mask adjusted until appropriate for the size of the aorta. Analysis then began with flow measurements. A flow plane is positioned along the centreline until at the appropriate level on the aorta. Each flow plane was positioned at the aortic root, proximal ascending aorta, middle ascending aorta, and distal ascending aorta in which measurements would be taken. Adjustments were made using double oblique views until cross-sectional images were accurately displayed and flow planes aligned. Each measurement was added, and flow calculation determined. Net flow (ml/cycle), Peak velocity (cm/s), and regurgitant flow (k) values were calculated automatically. Using the same anatomical plane, wall sheer stress was automatically calculated. Axial maximum WSS (Pa) and Axial average WSS (Pa) was determined.

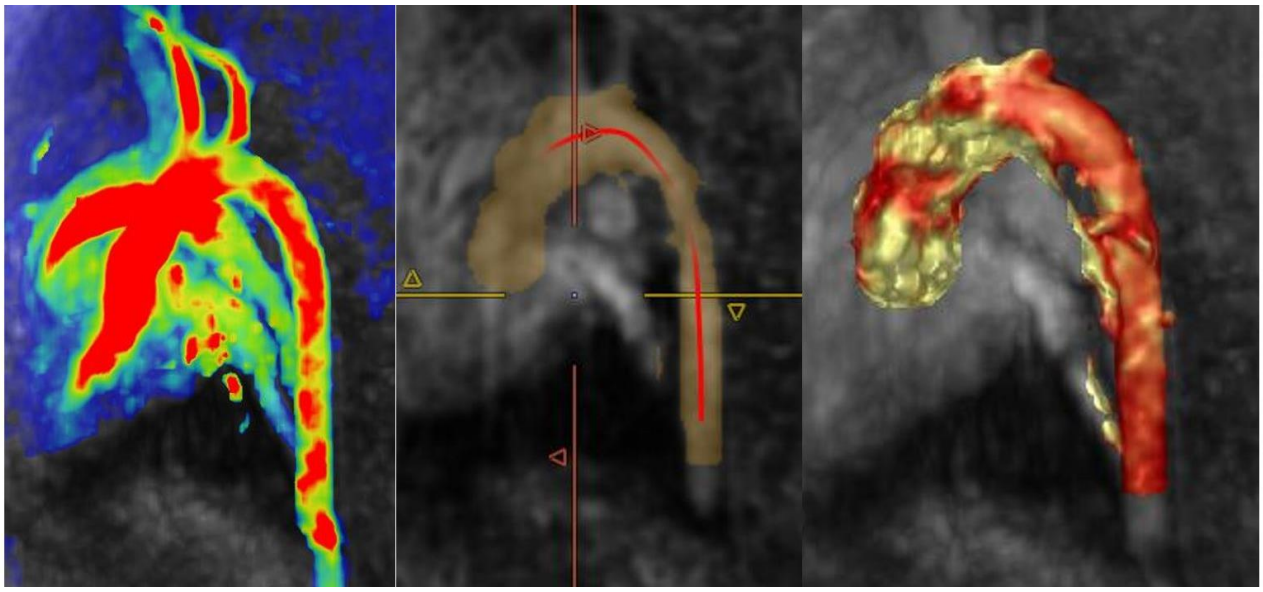

Figure 4.1: Pig subject 4D flow MRI pre-processing (left), segmentation (middle), and the aorta ready for analysis (right) as performed using Circle CVI42 version 5.10.1.

Siemens Skyra 3T 4D Phase Contrast Flow Parameters	
Field of view (FOV)	390mm x 266mm
Matrix	176 x 141
Voxel size	2.2mm x 2.2mm x 2.2mm (isotropic)
Repetition time (TR)	40.32ms
Echo time (TE)	2.29ms
Velocity encoding (VENC)	180
flip angle	8 degrees
Gating	Retrospective cardiac
Coils	Spine matrix and 18-channel body array

Table 4.2: MRI phase contrast flow parameters.

4.5.3 Animal operation

Following MRI imaging and normalization of pig haemodynamics including heart rate and blood pressure, a cardiopulmonary bypass circuit was created to replicate an adult circuit with a cardiac perfusionist managing its function. Two veterinary assistants monitored and managed the pig throughout the process. Two surgeons were the primary operators for each pig. For all experiments, cardiopulmonary bypass (LivaNova Circuit) was utilized, prepared with a roller pump, and inspire oxygenator.

Three-eighths tubing was used to replace the pump header, attached to the autolog reservoir, and inspire cardiotomy reservoir and clamped off. Two suckers were utilised for the operative field and

primed with 25,000 IU of heparin in 1000 ml of saline. The CPB circuit was primed with 1.6L of saline and 5000 IU of heparin. Operation time for each pig was between 60–120 minutes. Direct anterior access via a median sternotomy proved to give best access to the aorta and right atrium for cannulation (Figs. 4.2 and 4.3). Following heparinisation of 15,000 IU, the right atrium was cannulated using a 32f Medtronic venous canula, and the ascending aorta cannulated with a 16f Edwards Lifesciences cannula. Bypass was initiated with good flows, with incremental increases in pressures over the next 10 minutes. A cross clamp was applied at the distal arch. Cardiopulmonary bypass flows were then increased to maximal flows (L/min) and line pressures, and kept at these measures for 60 seconds with ongoing monitoring until aortic or cardiac failure. Cardiopulmonary bypass was ceased, and euthanasia was performed with 20 ml of intravenous phenobarbitone overdose.

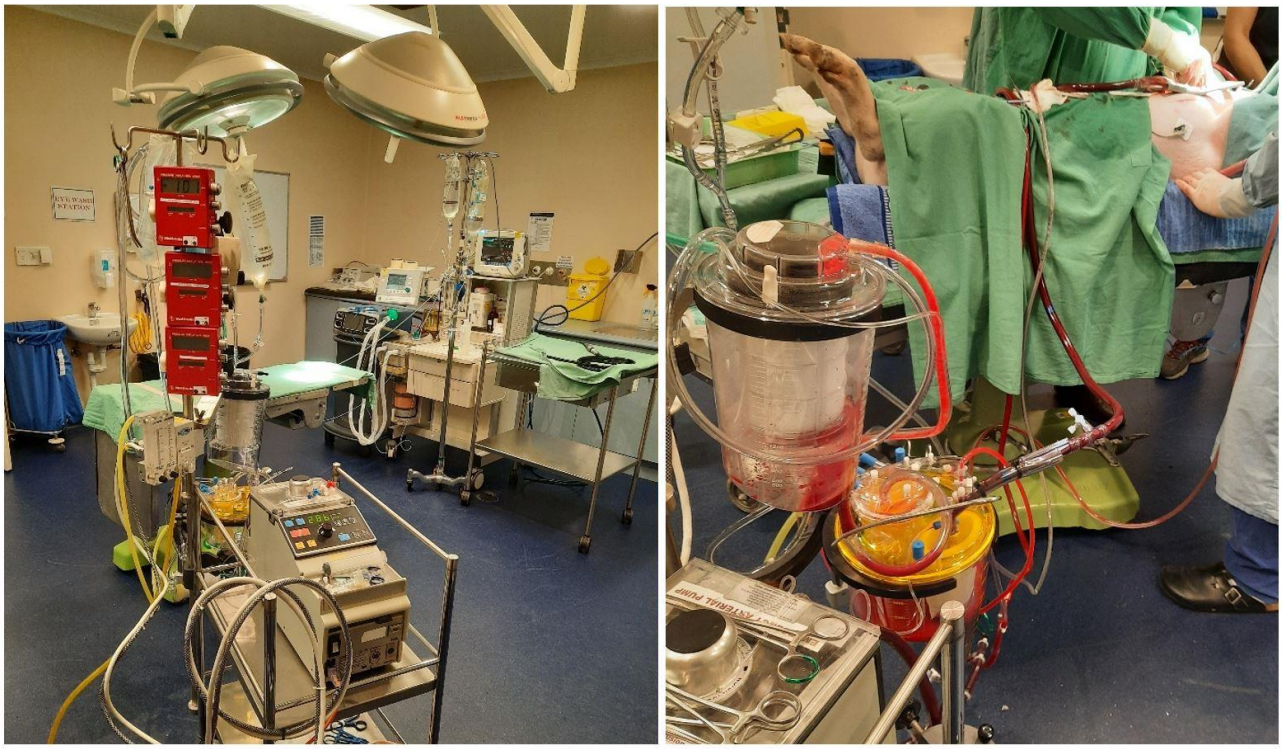

Figure 4.2: Cardiopulmonary bypass circuit setup for pig testing (left), and the active CPB circuit during the pig experiments (right).

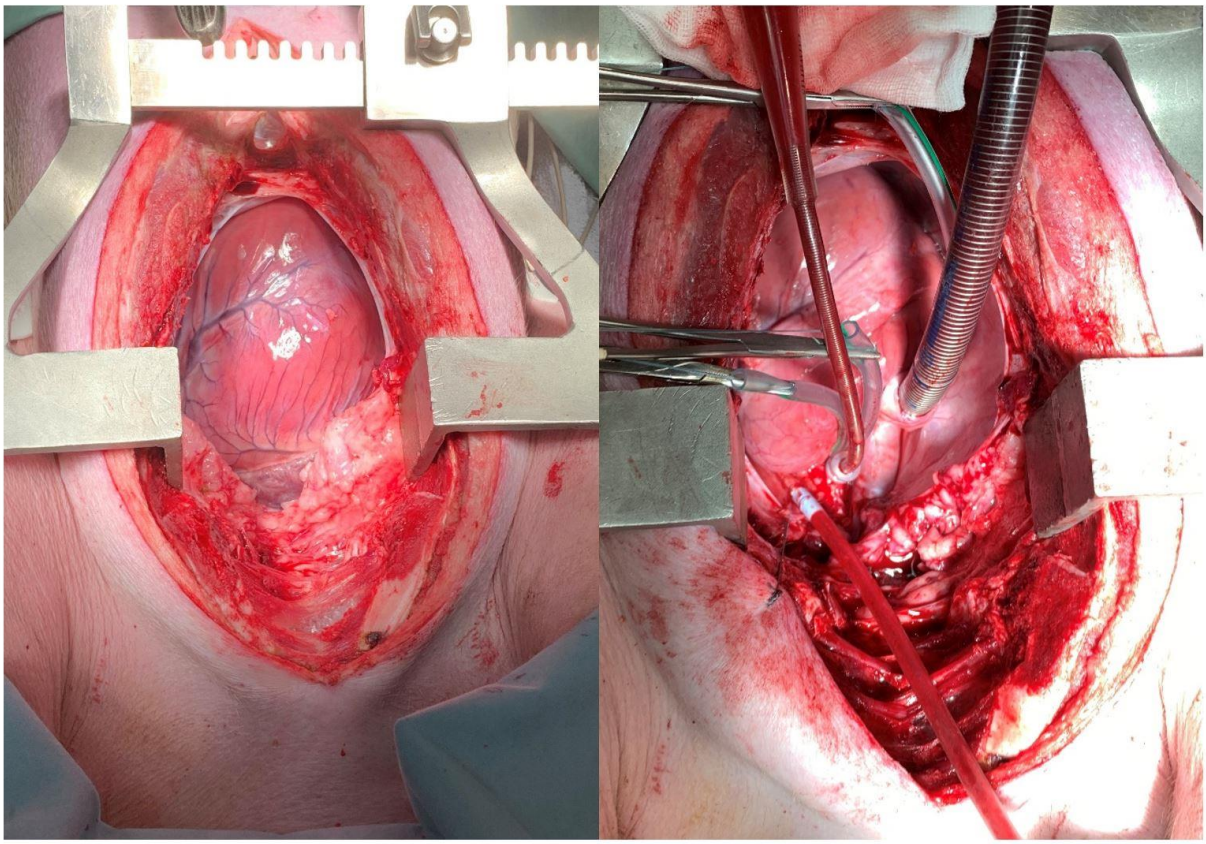

Figure 4.3: Median sternotomy and pig heart exposed (left), and establishment of central cardiopulmonary bypass with pig subject (right).

4.5.4 Macroscopic and histological analysis

The aorta was carefully dissected from the left ventricle to the start of the aortic arch in all pigs. Careful attention was made to handling the aorta to ensure no tissue damage was inflicted in this process. The aortic root, and ascending aorta were then cut into aortic root, proximal, mid, and distal regions and examined by the two operating surgeons.

Tissue was immediately placed in formalin for fixation following preparation, embedded, and cut using a Leica rotary microtome (Leica Biosystems, Mt Waverley Australia) into 5micro-metre edge-to-edge sections. The basic histological stains and special stains used included Hematoxylin and Eosin (H&E), Van Gieson (EVG), and Massons Trichrome (Massons), Alcian blue, and Von Kossa (VK) stains. Specific immunochemistry antibodies staining for Collagen type I, III and IV were obtained from Abcam Australia Pty Ltd (Melbourne, Victoria, Australia). Anti-Collagen I antibody, Anti-Collagen III antibody, and Anti-Collagen IV antibody were sourced.

Observational analysis proceeded with the primary investigator and a clinical histopathologist. Histological analysis occurred with the use of a double headed microscope at the University of Adelaide Histological department, Adelaide, South Australia.

Histological slides were scanned using Nanozoomer digital slide scanner (Hamamatsu Photonics), Zen Blue 3.0 (Zeiss) and NDP view 2.0 (Hamamatsu Photonics) depending on the slide size. Scanned histological slides were then analysed using Fiji by Image J (National Institutes of Health, USA).

Quantification of elastin and collagen fibres then proceeded using the colour deconvolution plugin (IHC toolbox) in Image J v.1.53 (The University of Nottingham, UK). The image was imported into Image J from the NDP or Zen programs, the image cropped to select a region of interest (ROI), and then colour deconvoluted. This ROI then underwent analysis and measurement in Image J to produce a percentage quantification of collagen fibres or elastin fibres within that tissue specimen.

4.6 Results

4.6.1 Clinical results

The clinical results from the 5 pig studies are summarized in Table 4.3. Median maximal aortic pressures obtained amongst the tested samples was 497 mmHg. The median maximal CPB flows (L/min) was 3.96L. The most common macroscopic findings were aortic cusp haemorrhage and non-coronary cusp tearing which occurred in 4/5 samples (80% of tested cases).

Swine number	Surgical approach	Cannulation	CPB flows (L/min)	Maximal pressure	Macroscopic findings
1	Right thoracotomy	Arterial– ascending aorta Venous – Right atrium	2L	280mmHg	• Valvular failure with no evidence of cusp tearing • Cusp haemorrhage present • Superior Vena Cava (SVC) tearing resulting in exsanguination of subject
2	Median sternotomy	Arterial – ascending aorta Venous – right atrium	2.2L	286mmHg	• Non-coronary cusp tearing and valvular rupture • Cusp haemorrhage • Subject euthanized
3	Median sternotomy	Arterial – ascending aorta Venous – right atrium	4.3L	500mmHg	• Non-coronary cusp tearing and valvular rupture • Cusp haemorrhage • Subject euthanized

4	Median sternotomy	Arterial – ascending aorta Venous – right atrium	5.4L	505mmHg	• Non-coronary cusp tearing and valvular rupture • Subject euthanized
5	Median sternotomy	Arterial – ascending aorta Venous – right atrium	3.96L	497mmHg	• Non-coronary cusp tearing and valvular rupture • Cusp haemorrhage • Subject euthanized
Median			**3.96L**	**497mmHg**	

Table 4.3: Clinical results and macroscopic findings following maximal aortic pressures on CPB.

4.6.2 Radiological results

The median max flow (cm/s) in all samples was 79.05 at baseline, and 95.53 following vasopressor. The median wall sheer stress (WSS) (Pa) in all samples was 0.31 at baseline, and 0.48 following vasopressor (Table 4.4).

Swine number	Region of aorta	Analysis number	Mean/Max Peak velocity pre vasopressor (cm/s)	Mean/Max peak velocity post vasopressor (cm/s)	Mean/Max WSS pre vasopressor (Pa)	Mean/Max WSS post vasopressor (Pa)
1	**Root**	**1**	79.83	52.41	0.09	0.28
		2	51.96	53.01	0.10	0.29
	Proximal Ascending Aorta	**1**	98.78	76.99	0.11	0.40
		2	54.54	83.04	0.08	0.40
	Middle Ascending Aorta	**1**	95.27	98.78	0.14	0.48
		2	77.52	96.62	0.11	0.45
	Distal Ascending Aorta	**1**	52.41	95.27	0.13	0.39
		2	110.26	102.21	0.13	0.42
2	**Root**	**1**	63.42	131.70	0.11	0.38
		2	54.02	65.91	0.26	0.38
	Proximal Ascending Aorta	**1**	74.53	146.69	0.21	0.49
		2	87.20	89.71	0.46	0.49
	Middle Ascending Aorta	**1**	99.92	184.53	0.31	0.75
		2	107.32	113.69	0.62	0.75
	Distal Ascending Aorta	**1**	102.02	186.72	0.35	0.57
		2	104.44	101.68	0.43	0.57
3	**Root**	**1**	52.37	62.32	0.20	0.26
		2	51.04	54.73	0.20	0.30
	Proximal Ascending Aorta	**1**	73.43	94.47	0.33	0.43
		2	74.93	88.95	0.31	0.45
	Middle Ascending Aorta	**1**	83.09	105.93	0.37	0.58
		2	88.32	98.03	0.40	0.58
	Distal Ascending Aorta	**1**	78.34	99.95	0.24	0.32
		2	77.23	98.24	0.25	0.37
4	**Root**	**1**	52.85	67.45	0.33	0.46

		2	53.78	59.31	0.32	0.40
	Proximal Ascending Aorta	**1**	69.98	88.21	0.50	0.67
		2	70.11	66.38	0.51	0.64
	Middle Ascending Aorta	**1**	82.58	93.25	0.62	0.82
		2	81.45	88.99	0.60	0.81
	Distal Ascending Aorta	**1**	95.40	102.49	0.55	0.80
		2	94.40	102.78	0.55	0.82
5	**Root**	**1**	86.29	75.83	0.28	0.31
		2	72.25	77.78	0.39	0.55
	Proximal Ascending Aorta	**1**	84.10	85.03	0.44	0.60
		2	96.32	95.79	0.31	0.61
	Middle Ascending Aorta	**1**	86.31	109.12	0.37	0.60
		2	79.73	103.87	0.27	0.55
	Distal Ascending Aorta	**1**	56.38	101.28	0.26	0.48
		2	50.54	102.78	0.43	0.37
	Median		**79.04**	**95.53**	**0.31**	**0.48**

Table 4.4: Summary of radiological results following noradrenaline administration and 4D flow MRI imaging.

The median max flow (cm/s) at baseline in the aortic root was 53.90, and 64.12 following vasopressor. Median flow in the proximal ascending aorta at baseline was 74.73 and 88.58 following vasopressor. Median flow in the middle ascending aorta at baseline was 84.70 and 101.33 following vasopressor. Median flow in the distal ascending aorta at baseline was 86.37, and 101.95 following vasopressor (Figure 4.4) (Table 4.5 and 4.6).

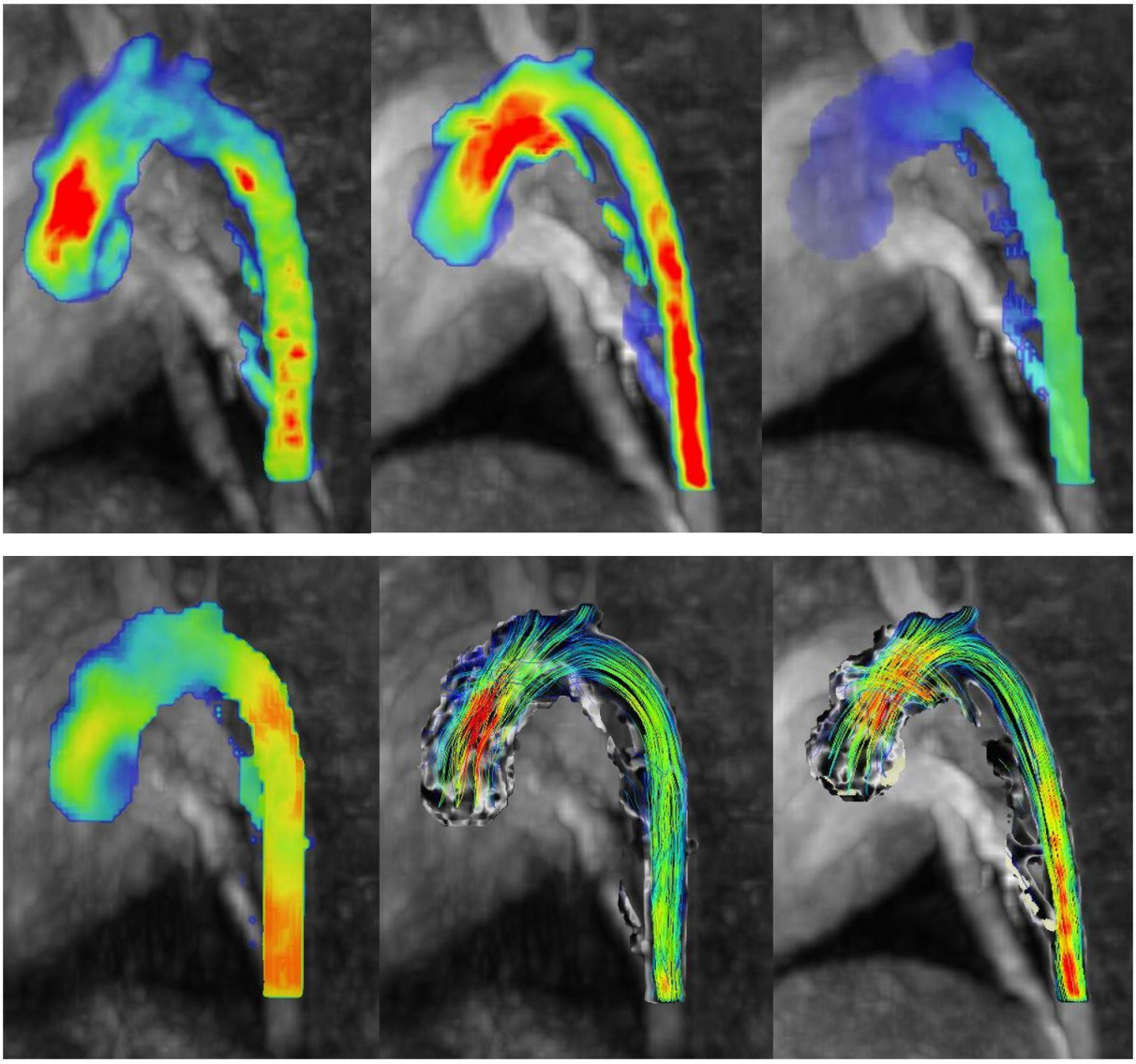

Figure 4.4: 4D flow MRI imaging results in the pig subjects. Top left – Pig flow measurements pre-administration of noradrenaline and Top centre – pig flow measurements post-administration of noradrenaline. The red shading indicates areas of higher flow measurements (cm/s). Pig wall sheer stress measurements. Top right – pig WSS measurements pre-administration of noradrenaline and Bottom left – pig WSS measurements post-administration of noradrenaline. The areas of yellow-orange-red identify regions of higher WSS (Pa) in ascending order in the pig subject. Bottom centre – Pig path line results pre-administration of noradrenaline and Bottom right – pig path line results post-administration of noradrenaline. The path lines show the direction of blood flow during these stages.

Subject number	Root	Proximal	Middle	Distal
1	79.83	98.78	95.27	52.41
2	51.96	54.54	77.52	110.26
3	63.42	74.53	99.92	102.02
4	54.02	87.2	107.32	104.44
5	52.37	73.43	83.09	78.34
6	51.04	74.93	88.32	77.23
7	52.85	69.98	82.58	95.4
8	53.78	70.11	81.45	94.4
9	86.29	84.1	86.31	56.38
10	72.25	96.32	79.73	50.54
Median	**53.90**	**74.73**	**84.70**	**86.37**

Table 4.5: Regional analysis of Flow (cm/s) in 4D flow analysis pre-vasopressor administration.

Subject number	Root	Proximal	Middle	Distal
1	52.41	76.99	98.78	95.27
2	53.01	83.04	96.62	102.21
3	131.7	146.69	184.53	186.72
4	65.91	89.71	113.69	101.68
5	62.32	94.47	105.93	99.95
6	54.73	88.95	98.03	98.24
7	67.45	88.21	93.25	102.49
8	59.31	66.38	88.99	102.78
9	75.83	85.03	109.12	101.28
10	77.78	95.79	103.87	102.78
Median	**64.12**	**88.58**	**101.33**	**101.95**

Table 4.6: Regional analysis of Flow (cm/s) in 4D flow analysis post vasopressor administration.

The median WSS (Pa) at baseline in the aortic root was 0.23, and 0.35 following vasopressor. Median WSS in the proximal ascending aorta at baseline was 0.32 and 0.49 following vasopressor. Median WSS in the mid ascending aorta was 0.37 at baseline, and 0.59 following vasopressor. Median WSS in the distal ascending aorta was 0.31 at baseline, and 0.45 following vasopressor (Table 4.7 and 4.8). Although not a direct measure within our study cohort, observational analysis of path lines pre- and post-administration of vasopressor showed increased vortices flow within the ascending aorta following the administration of vasopressor (Fig. 4.4).

Subject number	Root	Proximal	Middle	Distal
1	0.09	0.11	0.14	0.13
2	0.1	0.08	0.11	0.13
3	0.11	0.21	0.31	0.35
4	0.26	0.46	0.62	0.43
5	0.2	0.33	0.37	0.24
6	0.2	0.31	0.4	0.25
7	0.33	0.5	0.62	0.55
8	0.32	0.51	0.6	0.55
9	0.28	0.44	0.37	0.26
10	0.39	0.31	0.27	0.43
Median	**0.23**	**0.32**	**0.37**	**0.31**

Table 4.7: Regional analysis of WSS (Pa) in 4D flow analysis pre-vasopressor administration.

Subject number	Root	Proximal	Middle	Distal
1	0.28	0.4	0.48	0.39
2	0.29	0.4	0.45	0.42
3	0.38	0.49	0.75	0.57
4	0.38	0.49	0.75	0.57
5	0.26	0.43	0.58	0.32
6	0.3	0.45	0.58	0.37
7	0.46	0.67	0.82	0.8
8	0.4	0.64	0.81	0.82
9	0.31	0.6	0.6	0.48
10	0.55	0.61	0.55	0.37
Median	**0.35**	**0.49**	**0.59**	**0.45**

Table 4.8: Regional analysis of WSS (Pa) in 4D flow analysis post-vasopressor administration.

4.6.3 Histological results

Large tears beneath the non-coronary cusp were noted in all samples (Fig. 4.5).

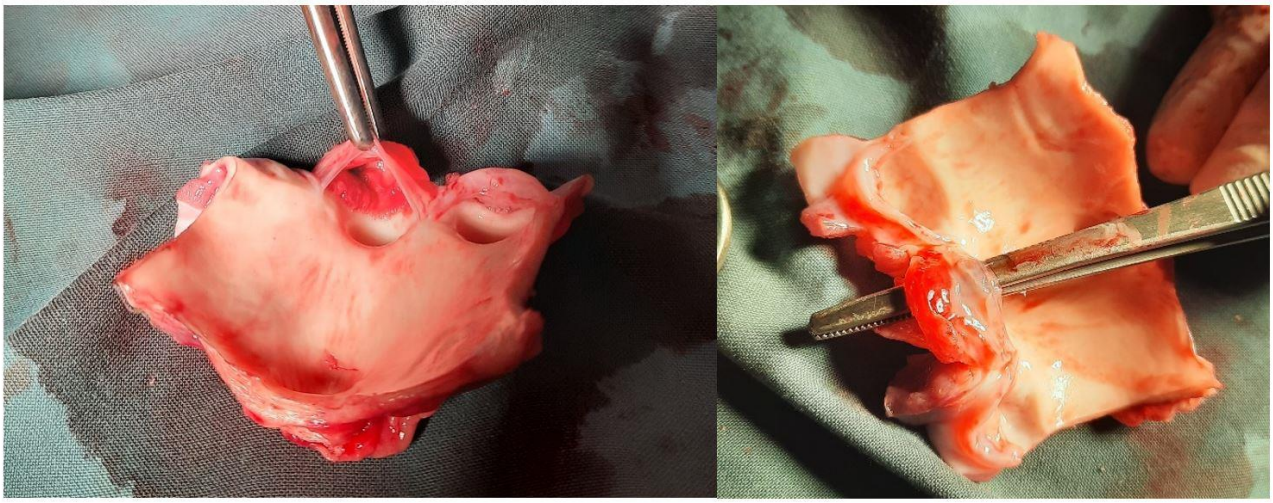

Figure 4.5: Photographs of the excised and opened aortic root identifying the tears beneath the non-coronary cusp within each pig subject tested (Experiment 2-5).

The average collagen composition (%) was highest in the proximal inner region (8.48) and proximal outer region (9.08); with other regions having approximately half that of the proximal regions. The average elastin composition (%) was highest in the proximal inner region (23.10). Elastin content was also high in distal and middle inner regions and the aortic root itself compared to other regions (Tables 4.9 and 4.10) (Fig. 4.6).

Swine number	Tissue sampled	Distal anterior	Distal posterior	Distal inner	Distal outer	Middle anterior	Middle posterior	Middle inner	Middle outer	Proximal anterior	Proximal posterior	Proximal inner	Proximal outer	Aortic root
1(1)	Ascending aorta and aortic root	2.49	7.20	0.17	1.25	11.61	2.42	5.47	2.28	1.82	3.99	6.17	12.44	1.83
1(2)	Ascending aorta and aortic root	3.21	7.23	1.13	1.28	10.56	2.67	5.98	3.56	1.98	4.85	7.24	10.34	1.98
2(1)	Ascending aorta and aortic root	3.08	1.50	7.65	2.18	3.78	5.82	0.84	4.36	2.33	4.76	9.24	9.65	0.87
2(2)	Ascending aorta and aortic root	3.77	1.55	7.22	2.56	3.88	5.99	1.23	4.44	2.57	4.65	8.88	9.02	1.06
3(1)	Ascending aorta and aortic root	3.33	5.55	1.27	2.01	4.06	5.03	5.25	3.81	2.34	4.32	7.32	9.84	3.77
3(2)	Ascending aorta and aortic root	4.56	5.06	1.85	2.32	4.32	5.55	5.91	4.00	2.21	4.74	9.35	9.13	3.02

4(1)	Ascending aorta and aortic root	3.75	6.00	2.75	2.67	3.72	6.78	5.12	4.76	2.56	4.54	8.55	8.34	2.94
4(2)	Ascending aorta and aortic root	3.75	6.11	2.69	2.11	3.91	5.10	5.87	4.12	2.75	3.87	8.56	8.75	2.64
5(1)	Ascending aorta and aortic root	4.01	5.86	1.67	3.64	2.54	6.10	5.54	4.87	2.33	4.67	8.41	8.88	1.06
5(2)	Ascending aorta and aortic root	4.44	5.32	1.85	2.86	2.50	5.09	4.91	4.67	1.97	5.34	7.46	9.03	1.87
Median		**3.75**	**5.71**	**1.85**	**2.25**	**3.90**	**5.33**	**5.36**	**4.24**	**2.33**	**4.66**	**8.48**	**9.08**	**1.93**

Table 4.9: Collagen composition within the sampled tissues via colour deconvolution measurements.

Swine number	Tissue sampled	Distal anterior	Distal posterior	Distal inner	Distal outer	Middle anterior	Middle posterior	Middle inner	Middle outer	Proximal anterior	Proximal posterior	Proximal inner	Proximal outer	Aortic root
1(1)	Ascending aorta and aortic root	9.49	14.29	28.08	17.97	13.15	10.04	32.36	18.67	12.68	15.04	21.17	24.33	16.62
1(2)	Ascending aorta and aortic root	10.07	14.98	27.65	15.86	13.07	10.86	32.96	17.56	11.85	15.45	22.56	23.31	15.89
2(1)	Ascending aorta and aortic root	5.53	18.82	15.50	27.49	7.52	14.83	16.71	18.65	25.17	11.79	27.03	16.58	13.17
2(2)	Ascending aorta and aortic root	7.65	17.99	14.78	28.76	8.01	16.94	15.98	19.64	23.96	11.76	26.95	17.22	12.56
3(1)	Ascending aorta and aortic root	9.62	14.16	16.36	25.47	12.37	11.74	27.36	19.37	14.37	14.53	21.63	17.53	20.87
3(2)	Ascending aorta and aortic root	10.25	15.47	15.78	18.36	11.74	13.85	26.78	18.52	13.15	15.64	21.11	19.55	19.67
4(1)	Ascending aorta and aortic root	11.24	15.64	13.65	18.33	13.01	13.74	20.11	17.55	13.43	15.33	23.64	20.11	34.04
4(2)	Ascending aorta and aortic root	13.42	14.65	15.56	18.24	15.41	13.11	21.01	17.00	12.64	16.22	24.54	20.42	33.24
5(1)	Ascending aorta and aortic root	12.15	14.33	23.43	19.42	14.33	15.43	19.53	18.43	12.07	16.31	24.22	21.64	26.76
5(2)	Ascending aorta and aortic root	13.44	14.52	23.53	17.43	13.94	14.23	19.45	18.55	12.11	17.54	21.11	23.63	26.89
Median		**10.16**	**14.82**	**16.07**	**18.35**	**13.04**	**13.80**	**20.56**	**18.54**	**12.92**	**15.39**	**23.10**	**20.27**	**20.27**

Table 4.10: Elastin composition within the sampled tissues via colour deconvolution measurements.

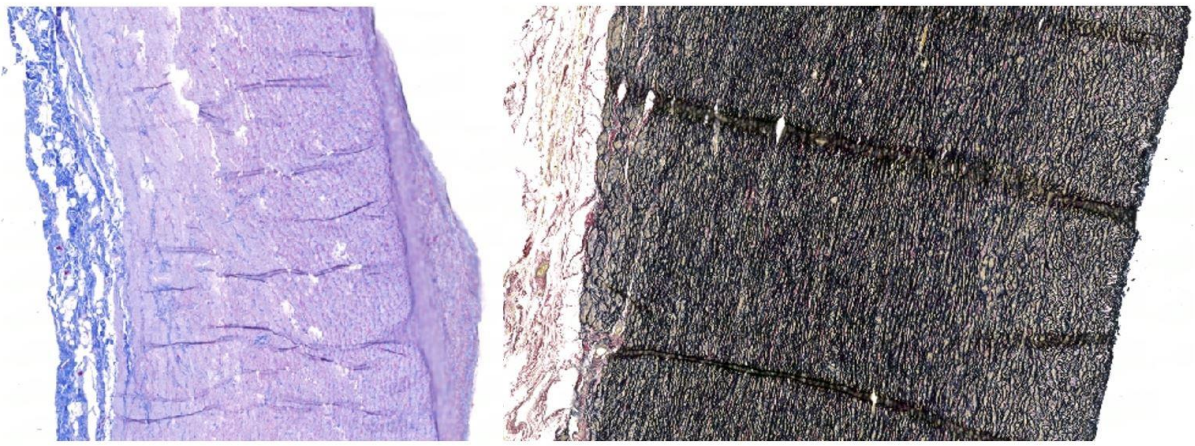

Figure 4.6: 10x Massons trichrome staining of the pig aortic root with darker blue areas indicating collagen deposition (left image). 10x Van Gieson (EVG) staining of the pig aortic root with black areas indicating elastin deposition (right image).

General observations were loss of tissue architecture in the aortic root and microhaemorrhages in the non-coronary cusp region in all subjects. Immunohistochemistry observations of Collagen I stained specimens showed stronger staining under the intimal layer in all subjects. Collagen III analysis showed diffuse and weak staining in all subjects. Collagen IV analysis showed gross staining with positive blood vessel internal markers within the aortic root in all specimens (Fig. 4.7).

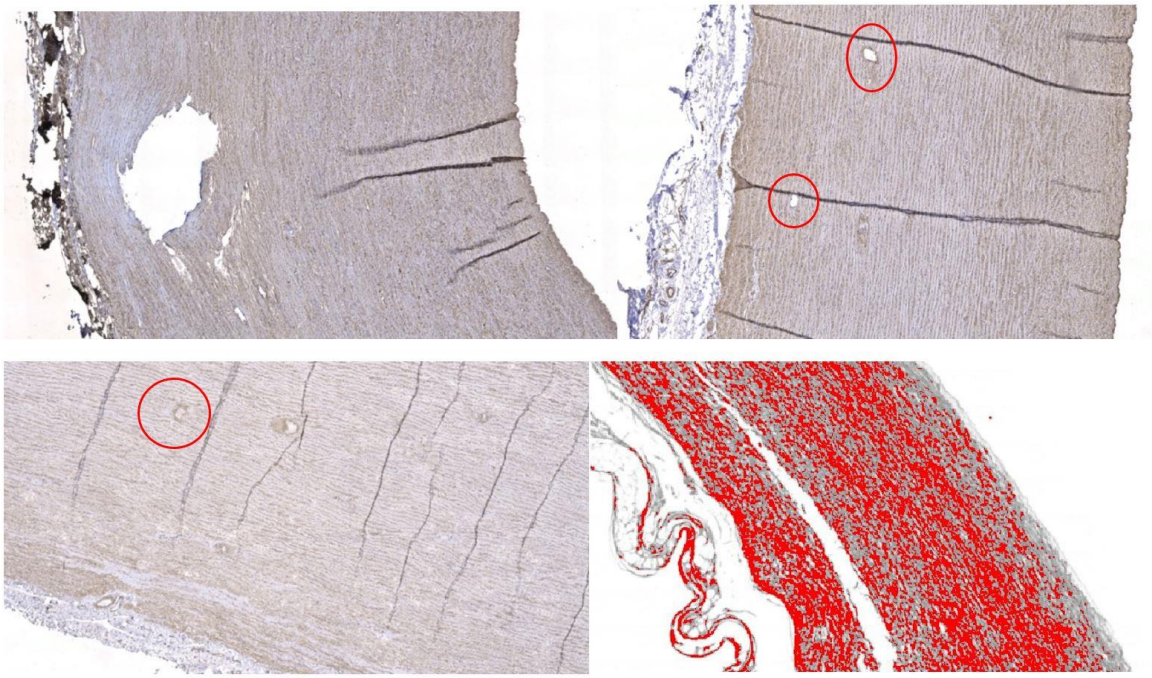

Figure 4.7: Immunohistochemistry results showing collagen types within the pig aortic root and ascending aorta. Top left – Collagen I stain within the pig proximal aorta as indicated by the brown staining. Top right - Collagen IV antibodies within the pig ascending aorta noting the positive internal structure staining of blood vessels as highlighted. Bottom left - Collagen IV antibodies within the pig ascending aorta with positive staining of internal blood vessels as highlighted. Bottom right - Colour deconvolution of immunohistochemistry results showing quantification of Collagen I in the proximal pig aorta as highlighted by the dense red areas.

The average collagen I composition (%) was highest in the distal inner region (28.92), followed by the middle inner (26.15), and proximal outer (25.75) regions. Collagen I was also high in the aortic root (24.53). The average collagen III composition (%) was highest in the middle inner (25.29) and aortic root regions (23.68). The median collagen IV composition (%) was highest in the middle outer (24.51) and proximal anterior (22.35) aorta (Tables 4.11-4.13).

Swine number	Tissue sampled	Distal anterior	Distal posterior	Distal inner	Distal outer	Middle anterior	Middle posterior	Middle inner	Middle outer	Proximal anterior	Proximal posterior	Proximal inner	Proximal outer	Aortic root
1(1)	Ascending aorta and aortic root	16.04	12.12	27.87	23.20	19.53	19.73	24.14	8.31	12.01	11.64	26.54	25.64	22.93
1(2)	Ascending aorta and aortic root	15.00	13.11	26.87	22.87	20.64	20.63	24.07	10.63	12.11	13.21	25.22	25.86	23.52
2(1)	Ascending aorta and aortic root	14.84	10.28	42.56	30.60	16.84	16.60	26.33	27.99	13.70	14.49	20.61	23.58	25.20
2(2)	Ascending aorta and aortic root	14.88	11.95	41.52	31.82	15.74	18.76	26.04	26.78	13.06	14.92	21.53	22.67	24.53
3(1)	Ascending aorta and aortic root	17.32	13.63	33.33	22.53	19.80	20.03	26.02	25.93	12.03	12.36	22.28	22.82	23.52
3(2)	Ascending aorta and aortic root	17.63	14.65	34.11	24.54	19.33	21.49	26.26	25.29	12.83	12.20	26.27	21.68	23.32
4(1)	Ascending aorta and aortic root	16.31	12.66	23.01	19.72	15.01	20.38	25.28	28.02	11.46	13.27	27.27	27.57	25.83
4(2)	Ascending aorta and aortic root	16.33	13.83	22.02	19.55	16.22	20.10	27.02	28.93	11.44	15.47	28.10	29.37	24.53
5(1)	Ascending aorta and aortic root	15.00	12.52	27.92	19.21	19.03	16.81	27.25	18.30	13.10	12.17	16.81	27.94	26.42
5(2)	Ascending aorta and aortic root	15.92	12.44	29.92	19.42	18.11	19.11	27.22	18.92	12.26	12.71	18.81	27.24	26.53
Median		**15.98**	**12.59**	**28.92**	**22.70**	**18.57**	**19.88**	**26.15**	**25.61**	**12.19**	**12.96**	**23.75**	**25.75**	**24.53**

Table 4.11: Immunohistochemistry results reporting on the percentage of collagen types I in all tissue samples.

Swine number	Tissue sampled	Distal anterior	Distal posterior	Distal inner	Distal outer	Middle anterior	Middle posterior	Middle inner	Middle outer	Proximal anterior	Proximal posterior	Proximal inner	Proximal outer	Aortic root
1(1)	Ascending aorta and aortic root	15.13	10.15	21.31	16.93	11.73	7.50	35.25	21.62	10.60	6.79	16.80	15..59	23.35
1(2)	Ascending aorta and aortic root	16.93	11.35	20.91	16.29	11.10	6.35	33.26	21.84	10.75	7.00	16.02	14.54	22.83
2(1)	Ascending aorta and aortic root	16.39	10.84	18.47	16.02	10.35	10.35	16.24	20.54	11.45	8.30	15.50	16.53	20.40
2(2)	Ascending aorta and aortic root	16.29	13.29	19.51	15.37	11.47	10.47	17.13	19.36	10.30	9.80	16.35	15.20	24.01
3(1)	Ascending aorta and aortic root	17.53	11.34	18.82	18.83	12.42	10.54	25.24	23.47	9.46	7.60	15.30	11.54	26.01
3(2)	Ascending aorta and aortic root	17.49	10.39	18.94	18.01	12.34	10.20	25.35	22.54	9.32	7.24	15.01	11.04	25.22
4(1)	Ascending aorta and aortic root	20.74	10.43	19.32	17.81	17.13	13.86	25.34	17.42	11.46	6.76	18.54	12.00	24.22
4(2)	Ascending aorta and aortic root	19.83	11.34	23.28	18.72	16.53	11.74	26.86	15.57	12.30	6.06	17.22	12.10	19.34
5(1)	Ascending aorta and aortic root	17.81	12.93	23.10	16.73	15.42	12.03	23.46	23.54	10.30	6.90	13.30	15.64	28.02
5(2)	Ascending aorta and aortic root	15.91	11.49	18.92	15.38	15.90	10.82	24.98	23.67	10.55	6.03	13.20	15.39	22.05
Median		**17.21**	**11.34**	**19.42**	**16.83**	**12.38**	**10.51**	**25.29**	**21.73**	**10.58**	**6.95**	**15.76**	**14.54**	**23.68**

Table 4.12: Immunohistochemistry results reporting on the percentage of collagen types III in all tissue samples.

Swine number	Tissue sampled	Distal anterior	Distal posterior	Distal inner	Distal outer	Middle anterior	Middle posterior	Middle inner	Middle outer	Proximal anterior	Proximal posterior	Proximal inner	Proximal outer	Aortic root
1(1)	Ascending aorta and aortic root	16.39	9.17	13.21	13.29	12.00	28.70	22.49	26.00	22.21	19.32	16.34	19.50	15.97
1(2)	Ascending aorta and aortic root	16.03	10.24	13.21	13.35	11.89	27.39	21.54	25.00	23.42	18.30	16.23	18.03	15.13
2(1)	Ascending aorta and aortic root	15.46	11.64	14.20	14.42	13.52	24.75	20.30	24.56	17.34	23.34	13.01	19.34	16.42
2(2)	Ascending aorta and aortic root	15.04	11.72	15.20	14.13	13.20	23.03	24.43	23.50	18.32	22.54	17.39	18.43	15.32
3(1)	Ascending aorta and aortic root	16.42	10.45	13.02	17.13	11.56	22.53	22.43	24.46	25.23	22.83	16.01	15.89	18.32

3(2)	Ascending aorta and aortic root	16.12	10.20	14.20	16.5	11.39	24.03	23.11	24.56	22.49	21.02	16.40	16.74	14.24
4(1)	Ascending aorta and aortic root	18.50	13.23	13.02	11.23	12.03	26.04	22.03	24.56	21.23	19.34	15.03	19.34	15.63
4(2)	Ascending aorta and aortic root	19.42	13.10	14.93	11.00	12.20	25.94	19.44	24.09	28.34	34.39	15.30	19.00	14.24
5(1)	Ascending aorta and aortic root	14.24	11.50	14.20	15.20	12.56	25.38	20.79	24.40	22.54	33.32	14.03	18.42	16.40
5(2)	Ascending aorta and aortic root	14.10	12.39	13.20	15.60	12.57	25.11	22.20	22.03	22.12	21.20	16.03	21.32	17.22
Median		**16.10**	**11.57**	**14.17**	**14.28**	**12.12**	**25.26**	**22.12**	**24.51**	**22.35**	**17.85**	**16.02**	**18.72**	**15.80**

Table 4.13: Immunohistochemistry results reporting on the percentage of collagen types IV in all tissue samples.

4.7 Discussion

The aim of this study was to determine which area of the aortic root was most susceptible to failure at high aortic pressures, and how these pressures manifest radiologically and histologically in acute rupture. Using a physiological model, we identified patterns of radiological, pathological, and histological change in the aorta under pressure. Although there are many studies demonstrating the effectiveness of the pig cardiopulmonary bypass model and its relevance to the human situation [4–13], only Surman et al. [1] has demonstrated the effect of maximal aortic pressures on the aortic root and ascending aorta in this model.

Intimal tears are reported to occur mostly in the right lateral wall of the ascending aorta in humans [3], however studies reporting on the most common sites are not well described. Tears affecting the proximal ascending aorta and distal arch have the most catastrophic consequences as they compromise the heart, and brain, respectively. Although our pig subjects were not aneurysmal, not all dissections and aortic rupture occurs in aneurysmal patients, and therefore the results hold pathological value in interpretation. When it came to location of the tears, all pig subjects had splitting beneath the noncoronary cusp and aortic valve failure, identifying it as an area of weakness under high continuous aortic stresses. Surman and colleagues [1] found that the aortic root apparatus in pig subjects failed at lower pressures compared to the ascending aorta, identifying a clear difference between these two tissues. Clinical findings in this study, supported those findings with failure of the aortic valve apparatus and preservation of the ascending aorta in all regions.

We examined the impact of high intraluminal pressures on the aorta using 4D flow MRI. Median flow measured in cm/s increased significantly, and WSS almost doubled on average across all

subjects in all regions of the aorta, identifying that high stresses manifest throughout the aorta from root to distal ascending in only an acute period. When we examine the regional changes, the proximal, middle, and distal ascending aorta had significant increase in flow following vasopressor administration indicating that this distribution of increased flow propagates from the root to the arch. Even more profound, was that WSS (Pa) almost doubled in all regions of the aorta following vasopressor administration. The increase in aortic stress was greatest in the mid ascending aorta but high in all regions from the root to the arch. The increase in WSS also correlates to the WSS showing highest increases in the mid and distal ascending aorta groups.

When we review the acute immunohistochemistry and histological changes that result from these acute stresses, we must determine what is normal before comparing to what is abnormal. The two main types of collagens found in the aorta are types I and III and account for 80–90% of the total collagen, and remaining collagens in lesser amounts [14]. Collagen staining of types I and III was more intense in cases of thoracic ascending aorta dissection than in controls and were characterized by thick longitudinal sheets or bundles in the media which were larger than type IV [14, 15], while others show collagen proportion in the wall of the dissected and aneurysmal thoracic ascending aorta was less than control [16, 17]. Histological and immunohistochemistry analysis in a swine model is not reported in the literature. Interestingly we found that Collagen type I had quite intense staining throughout the intimal layers in all specimens, whereas type III was less abundant. Type IV collagen is less abundant but in control ascending aorta and normal histological samples of ascending aorta dissection, type IV collagen were seen between the subintimal basement membrane and the media, and in the basement membrane of the adventitia [14].

In our study, collagen IV was prominent in the proximal ascending and aortic root compared to other regions. Eckhouse and colleagues [6] in thoracic abdominal aneurysms in pigs, reported aortic structural changes including elastic lamellar degradation and decreased collagen content. and colleagues [18] examined differences in aortic sinus tissues between human and pigs. The pig tissues contain a higher proportion of elastin than the human tissues, which contain a higher proportion of collagen. The elastin fibres in the pig tissues also appeared to be more undulated than the elastin fibres in the human samples, which were thinner and straighter. This study is limited by the use of a single special stain and lack of quantification of their findings. Collagen I was clearly higher within inner regions across proximal, middle, and distal aortic areas, and similarly collagen III was highest within inner regions including the aortic root. Collagen IV as the least commonly reported type in the thoracic aorta was more equally distributed across regions but showed some higher content in the more middle and proximal regions of the ascending aorta.

Determining protein quantification in pig tissue is scarce in the literature. A study in 1985 from Davidson and colleagues [19] aimed to determine this in newborn pigs. Relative collagen and elastin syntheses, as a per cent of total protein synthesis, were determined in four separate experiments. Elastin synthesis decreased from about 16.4% in the thoracic aorta to 1.6% of total protein

synthesis in the abdominal aorta. Collagen synthesis showed the opposite trend, increasing to 12% of total protein synthesis, although collagen synthesis was still a significant fraction (5–8%) of total protein synthesis in the upper thoracic tissue [19]. Collagen composition was reported as higher in the proximal inner and outer regions of our samples on average across all specimens. Elastin composition was also recorded highest in the inner regions across proximal, middle, and distal aortic regions.

This detailed live animal modelling under conditions of ongoing continuous flow have revealed some important information regarding acute aortic pathology. We have determined that area of greatest risk of failure during high pressure and flow conditions is the non-coronary cusp of the aortic valve within the aortic root apparatus as confirmed by macroscopic and microscopic findings. We have found that the regions of the thoracic ascending aorta under greatest WSS after increased vasopressor insult is the proximal and middle ascending aorta regions.

Histopathology analysis has revealed that the proximal and inner regions of the thoracic ascending aorta have collagen and elastin content that differs from the remaining aortic structure which may predispose or protect it from more chronic insults. When it came to specific collagen content as measured by immunohistochemistry, proximal and inner regions similarly had high collagen I, III, and IV levels but specifically the aortic root had some of the highest collagen I and III levels within the tested samples. We determined that Collagen IV was quite a dominant figure in the ascending aorta alone, but was found in minimal amounts in the aortic root.

When we compare the histological and immunohistochemistry analysis of non-aneurysmal samples in pigs and humans there are similarities between quantification values as reported in an upcoming article for publication by Surman and colleagues. When we compare human aneurysmal collagen and elastin quantities, the values are similar between human aneurysmal elastin content and pig elastin in this study, but the collagen content differs considerably. When we review the human aneurysmal immunohistochemistry versus pig values in this paper, we see significant differences. The quantity of Collagens I, III and IV are all significantly reduced in human aneurysms compared to non-aneurysmal acute ruptured pig samples. Reassuringly there is good reproducibility of quantification between pig and human nonaneurysmal samples as shown in earlier studies [4–13].

Limitations in this study includes histological analysis, whereby immunohistochemistry techniques are limited by the ability of the tissue to take up by the antibodies in question which result in more difficult specimens to analyse and quantify. In addition, pathological analysis and quantification are limited by the investigator and varies considerably with each analysis. Tables which show variation in final percentages following analysis by each investigator of the same sample. Limitations also include the surgical approach. Access and initiation of cardiopulmonary bypass is very challenging, and this was shown by difficulties in initial attempts at surgical access. The authors agree that a median sternotomy approach to the ascending aorta is best. Limitations in MRI include long

acquisition times, parallel imaging techniques used to compensate (i.e., decreased special and temporal resolution), and the sequence is a WIP so is still investigational.

To our knowledge, this is the first live pig study measuring the limits and resulting pathology of the aortic root and ascending aorta under high pressures during cardiopulmonary bypass supported by earlier pilot ex-vivo studies [1]. Similarly, no study has quantified the microscopic details of the aortic root and thoracic ascending aorta following such acute insult.

4.8 Conclusion

We have identified that the most vulnerable structure in the aortic root apparatus is the non-coronary cusp of the aortic valve. Furthermore, we have demonstrated that the aortic root has histopathological characteristics such as collagen content and collagen types that differ from the ascending aorta. This is supported by upcoming histological analysis of human subjects by Surman and colleagues.

These findings further support the idea that the aortic root apparatus, extending up to the proximal ascending aorta, needs to be considered as an independent structure within the aortic complex. Its unique structure, histology, and protein composition confer unique responses to pressure, and as such, the aortic root should be considered a more vulnerable and delicate structure than the other regions within the aorta. Further live animal testing in aneurysmal aortas may provide valuable additional data to build on the findings of the study.

4.9 References

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CHAPTER FIVE

Clinical outcomes in surgical and transcatheter aortic valve replacement. An ANZSCTS database review 2001-2019

Published in Heart Lung and Circulation Journal, May 2022

5.1 Statement of authorship

Statement of Authorship

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Name of Principal Author (Candidate)	Timothy Luke Surman		
Contribution to the Paper	Primary investigator and author Questionnaire completion Publication formulation		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	13/2/21

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	James Edwards		
Contribution to the Paper	Experimental design concept Publication planning		
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5.2 Manuscript summary

Title

Clinical outcomes in surgical and transcatheter aortic valve replacement. An ANZSCTS database review 2001-2019

Author's names and affiliations

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5.3 Abstract

Background

Since the last formal publication reporting on the findings of the Australian and New Zealand Society of Cardiac and Thoracic Surgeons (ANZSCTS) database on surgical aortic valve replacement (SAVR) and transcatheter aortic valve replacement (TAVR) in 2016, transcatheter approaches have become common practice. There has been an increase in use of TAVR following large, randomized control trials that only report on short term outcomes in a selective cohort. This study aims to report on primary outcome measures and identify complications associated with SAVR and TAVR from a large national database.

Methods

From the ANZSCTS database (2001-19), 14,097 SAVR and 1,194 TAVR patients were identified with clinical details and 30-day follow-up available. The primary endpoint was the composite of all-cause mortality and/or permanent stroke at 30 days. Secondary endpoints were post-procedure complications requiring treatment. Logistical regression followed by propensity score matching was performed.

Results

Using logistical regression when all patient factors considered for all patients who had SAVR and TAVR, the only preoperative factors that had an impact on 30-day mortality was cerebrovascular disease, respiratory disease, preoperative dialysis, angina, and hypertension. Primary outcome 30-day mortality rate was 1.83% in the SAVR group, and 1.68% in patients in the TAVR group, p=0.7001, and permanent stroke was seen in 1.07% patients in the SAVR group, and 1.26% patients in the TAVR group. Acute limb ischemia, aortic dissection, ventricular tachycardia, bradyarrhythmia and heart block were more common following TAVR ($p<0.001$), while reintubation and atrial arrhythmia were more common following SAVR ($p<0.001$).

Conclusions

In the real world SAVR and TAVR have been used in very different patient groups and it is difficult to compare as different baseline characteristics & complications. The two patient groups maintain similarities in primary and secondary endpoints, but differences in life threatening and life altering morbidity remains significant. Collection of SAVR and TAVR data in a combined database may help to better capture and compare these complications and institute strategies to prevent them.

5.4 Introduction

The surgical management of aortic stenosis (AS) with an aortic valve replacement (AVR) has been the evidence-based gold standard since 1961 when the first successful AVR was performed. Attempts using the ball-valve prosthesis by Harken and colleagues in 1960 [1] and Starr and Edwards in 1961 [2] resulted in high operative mortality, with 12.2% hospital deaths, and 26.5% total in-hospital and late deaths in 117 aortic valve patients [3]. Since 2002, transcatheter aortic valve replacement (TAVR) has become an evolving option in the management of aortic stenosis, and much of the debate and associated research regarding the management of AS has centred on SAVR versus TAVR.

Utilising 'real world data' from the ANZSCTS database, the primary objective of this study is to compare the 30-day outcomes (all-case death and permanent stroke) in patients undergoing SAVR and TAVR. Secondary objectives including comparing 30-day outcomes between these groups in relation to deep sternal wound infection and valvular dysfunction, and explore other early outcomes that greatly impact on patient morbidity and quality of life such as vascular complications and cardiac arrhythmias.

5.5 Materials and Methods

5.5.1 Study Population and Design

A comparative cohort study included institutions from 26 public and 32 private participating Cardiothoracic Surgical Units in Australia and New Zealand, including 20 Cardiothoracic Units that provided the TAVR data (Figure 5.1).

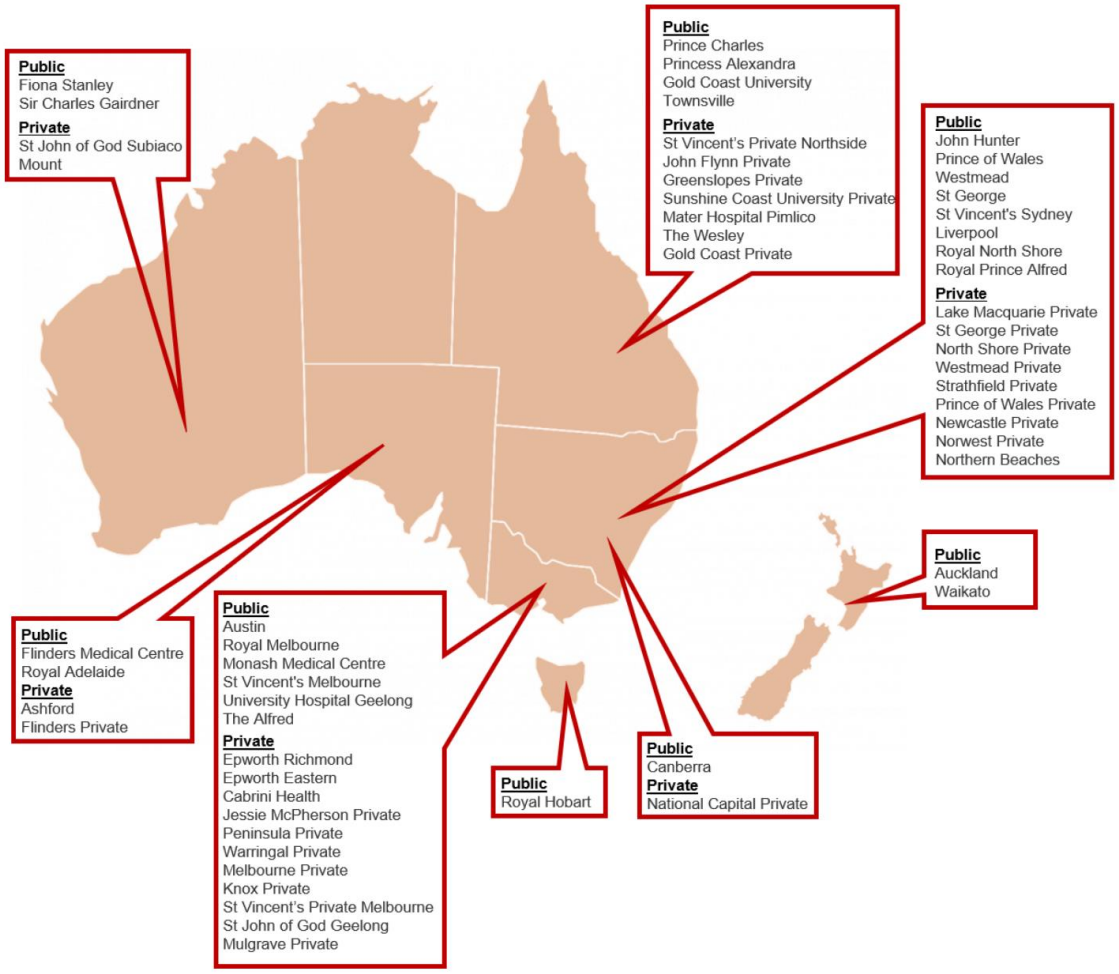

Figure 5.1: Participating public and private sites in the ANZSCTS database across Australia and New Zealand

Ethics and governance approval was obtained from The Central Adelaide Health Care Network (HREC/18/CALHN/188), with approval to utilize the ANZSCTS database remotely via The Safe Haven Environment at Monash University, Melbourne, Australia. From 2001-2019, a total of

15,291 patients were entered into the ANZSCTS database throughout Australia that underwent an AVR. Of these, 14,097 patients underwent SAVR, and 1,194 patients underwent TAVR (Figure 5.2)

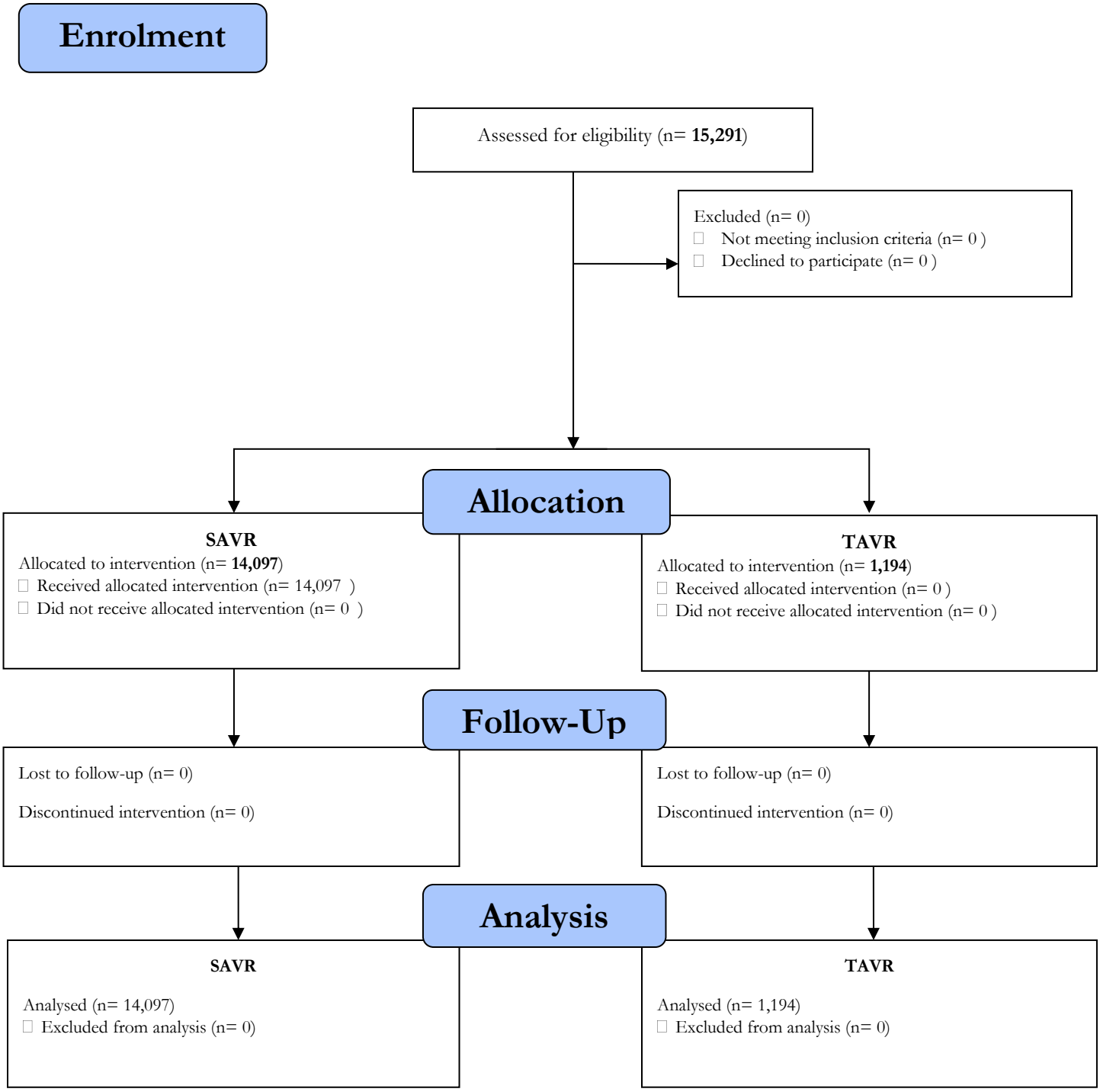

Figure 5.2: Consort diagram showing recruitment of participants for ANZSCTS cohort study.

The timeframes for the SAVR and TAVR cohorts were as follows. Data from the SAVR cohort was collected for cases with a date of procedure from the 4th of June 2001 to 31st December 2018. Data from the TAVR cohort was collected for cases with a date of procedure from the 16th of December 2008 to 20th of December 2018. The study period selected was those available for review from the completed database. There is bias in the data collected. Not all hospitals have submitted TAVR data, so it is not spread evenly across states. Variation may exist between surgeon-lead TAVR and cardiologist-lead TAVR, and therefore may not be representative of the full range of practice. Missing data was documented and confirmed as a % of the total cohort in the following postoperative complications; new renal failure (0.26% SAVR and 0.50% TAVR), permanent stroke (0.32% and 0.57% TAVR), pulmonary embolism (0.32% SAVR and 0.58% TAVR), Deep sternal wound infection (DSWI) (0.45% in SAVR, and 0.42% in TAVR), aortic dissection (3.43% in SAVR and 0.41% in TAVR), acute limb ischemia (3.43% in SAVR, and 0.41% in TAVR). There is no dropout data as patients are not followed up in this data collection process.

In addition to Australian data, we have reviewed the North American and German experiences with a focus on observational data and trends

5.5.2 Participant Selection

According to recommended practice by current United States and European guidelines [4], patients underwent SAVR and TAVR procedures after selection by the specific institution's patient recruitment process and heart team discussions. Most surgical patients would have been recruited via standard inpatient or outpatient referral, whereas transcatheter patients were likely to be selected following multidisciplinary heart team review. Patients are typically considered for TAVR if they were deemed to be of a higher operative risk and a transcatheter approach was deemed preferable over open surgical access. Inclusion and exclusion criteria were based on individual institutions guidelines and not protocol driven.

Data was utilized from the ANZSCTS database which records patient demographics, co-morbidities, procedure details, intraoperative data, and postoperative complications up to 30-days post procedure.

The North American experience began in 2010 with the Placement of Aortic transcatheter valves (PARTNER) I trial for patients who could not undergo aortic valve surgery. A total of 358 patients were enrolled across 21 centres in the United States, with 5-year outcomes being determined on 699 patients across the SAVR and TAVR high risk groups [5].

Amongst the intermediate risk groups in the PARTNER 2 trial, 2,032 patients were assigned across the two groups for comparison. In the low-risk PARTNER 3 trial, 1,000 patients were randomly assigned to both groups for comparison. The German experience in the aortic valve registry (German Aortic Valve Registry [GARY]) started reporting on outcomes following SAVR and TAVR patients from 1-year results in 13,860 very high-risk patients or inoperable patient [6], 7,613 intermediate risk patients [7], and 20,549 low risk patients [8] in 2019.

5.5.3 Procedure

Of the 14,097 patients who underwent SAVR, a bioprosthetic valve was used in 11,209 cases (79.5%), homo/allograft in 42 cases (0.3%), a mechanical valve was used in 2,675 cases (19%), and 107 cases were unknown (0.8%).

Of the 1,194 patients who underwent TAVR, the procedural access point was greatest via transfemoral access (536, 44.9%), transapical (46, 3.9%), transaortic (23, 1.9%), and trans subclavian (19, 1.6%). The valve types implanted are show below: A summary of TAVR valve types is in table 5.1.

Transcatheter valve type	**Total number of valves**	**% of valves in total cohort**
Sapien 3 9600TFX	401	33.6
CoreValve Evolut R	253	21.2
Sapien XT 9300	282	23.6
Portico valve	69	5.8
Sapien 3 S3TF1xx	42	3.5
Corevalve B	37	3.1
Sapien 9000	16	1.3
Evolut Pro	7	0.6
ACURATE neo SYM-SVxx-002	5	0.4
Boston Scientific Lotus Valve System – LTV27	4	0.3
Transapical B	1	0.1

Mechanical valve graft prosthesis	1	0.1
Unknown	8	0.7

Table 5.1: Transcatheter valve types used in TAVR cohort.

All patients in the SAVR and TAVR groups underwent valve replacement only. Cases with concurrent coronary bypass grafting, and other valve procedures were not included.

5.5.4 Study Endpoints

The primary study composite endpoints were 30-day all-cause mortality and permanent stroke persisting for > 72 hours peri or post-operatively. Secondary endpoints were:

a) Readmission for deep sternal wound infection within 30 days,
b) Readmission for valve dysfunction within 30 days,
c) New atrial arrhythmia (AF or flutter),
d) Heart block requiring implantation of PPM prior to discharge,
e) New ventricular tachycardia of > 6 beat run requiring treatment,
f) New renal insufficiency (characterized by >200mmol/0.2micromol/L increase and a doubling of the preoperative creatinine value or requiring hemofiltration or dialysis),
g) New pulmonary embolism diagnosed by ventilation/perfusion (V/Q scan) or CT angiogram
h) Continuous coma for > 24 hours in a nonsedated patient,
i) Pneumonia diagnosed by positive cultures of sputum/aspirate, and haematological or radiological evidence,
j) Aortic dissection,
k) Anticoagulation complications including bleeding, haemorrhage, and or embolic events related to anticoagulation,
l) Septicaemia defined as positive blood cultures and any two of fever, elevated granulocyte, elevated and increasing CRP, and elevated and increasing ESR postoperatively,
m) Acute limb ischemia,
n) Multi-organ dysfunction involving two or more major organ systems for > 48 hours,
o) GIT complications postoperatively including GI bleeding, pancreatitis, cholecystitis, ischemia, hepatitis, or other GI complication,
p) And re-intubation

5.5.5 Statistical analysis

Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software, San Diego, California). A p-value of <0.05 was considered significant. Comparisons between groups was determined using the N-1 Chi Squared test, which is deemed to have reduced type I errors and increased power compared to others, and is recommended where all expected numbers are at least 1, with t-test used for continuous variables. Logistical regression followed by propensity score matching was performed using SPSS. We performed a stepwise logistical regression analysis using all known patient preoperative demographics and co-morbidities that were collected as part of the ANZSCTS database. The dependent variable was 30-day mortality. Propensity matching was subsequently performed using the outcome of the logistical regression analysis with a tolerance of up to 1.

5.6 Results

Preoperative demographics, co-morbidities, and cardiac function of patients from the entire cohort are displayed in Table 5.2 and 5.3. The study endpoints are reported in table 5.4.

Patient variables	**SAVR n = 14097**	**TAVR n = 1194**	**p-value**
Mean Age			**P<0.001**
<60	2865 (20.3%)	10 (0.8%)	
60-70	3866 (27.4%)	50 (4.2%)	
71-80	5133 (36.4%)	275 (23.0%)	
81-90	2174 (15.4%)	859 (71.9%)	
>91	55 (0.4%)	108 (9.0%)	
Gender and ethnicity			
Male	8777 (62.3%)	666 (55.8%)	p <0.001
Female	5320 (37.7%)	528 (44.2%)	p <0.001
ATSI	190 (1.3%)	7 (0.6%)	p=0.0251
Mean BMI	29.48	27.6	p<0.0001
Mean BSA	1.87	1.77	P<0.0001
Mean valve size	26.66	23.42	P<0.0001
Minimum EoA (BSA x 0.85cm2/m2) to avoid PPM	1.59	1.5	P<0.0001

iEOA		0.84 **(Sapien 3 transcatheter valve), 1.12 (Evolut transcatheter valve)**	
NYHA			**P<0.001**
1	3260	85	
2	5467	332	
3	4427	669	
4	787	103	
Left Ventricular Ejection Fraction (LVEF)			
>60%	8570	596	p<0.0001
46-60%	3524	368	p<0.0001
30-45%	1204	178	p<0.0001
<30%	453	44	p=0.3776
Missing	346	8	
Re-do cardiac surgery	1972 (13.9%)	0 (0%)	p<0.0001
History of smoking	7151 (50.7%)	555 (46.5%)	p=0.0049
Missing or unknown	26 (0.2%)	31 (2.6%)	p<0.0001
Current smoker	1143 (8.1%)	32 (2.7%)	p<0.0001
Diabetes	3443 (24.4%)	392 (32.8%)	p<0.0001
Hypercholesterolemia	7609 (53.9%)	825 (69.1%)	p<0.0001
Preoperative dialysis	229 (1.6%)	32 (2.7%)	p=0.0069
Renal transplant	80 (0.6%)	5 (0.4%)	p=0.5069
Hypertension	9726 (69%)	1001 (83.8%)	p<0.0001
Cerebrovascular event	1453 (10.3%)	227 (19%)	p<0.0001
Remote CVA	657 (4.7%)	93 (7.8%)	p<0.0001
Recent CVA	84 (0.6%)	3 (0.3%)	p=0.1285
Peripheral vascular disease	816 (5.8%)	219 (18.3%)	p<0.0001

Respiratory disease	2147 (15.2%)	262 (21.9%)	p<0.0001
Infective endocarditis	947 (6.7%)	7 (0.6%)	p<0.0001
Active IE	670 (4.7%)	1 (0.1%)	p<0.0001
Family history CAD	1696 (12%)	65 (5.4%)	p<0.0001
Previous MI	1094 (7.7%)	228 (19.1%)	p<0.0001
CCF	4102 29.1%)	574 (48.1%)	p<0.0001

*Indexed EoA from Medtronic Inc (Sapien 3 transcatheter valve), 1.12 (Evolut transcatheter valve)

Table 5.2: Preoperative demographics and early postoperative outcomes of entire cohort. Definitions listed in study endpoint section*.

Cohort	**SAVR n= 14097**	**Average % of cohort**		**TAVR n = 1194**	**Average % of cohort**
Aortic valve size mm SAVR			**Aortic valve size mm TAVR**		
19	759	5.38	19	1	0.08
20	72	0.51	20	13	1.09
21	2969	21.06	21	-	-
22	94	0.67	22	-	-
23	4549	32.27	23	260	21.78
24	69	0.49	24	-	-
25	3562	25.27	25	9	0.75
26	105	0.74	26	441	36.93
27	1504	10.67	27	40	3.35
28	17	0.12	28	-	-
29	284	2.01	29	328	27.47
30	-	-	30	-	-
31	-	-	31	3	0.25
32	-	-	32	2	0.17
33	-	-	33	-	-
34	-	-	34	74	6.20
Total	13984			1171	
Missing	113*	0.80		23*	1.93
Mean valve size	**27mm**			**23mm**	

Table 5.3: Valve sizes in the SAVR and TAVR groups. Missing data was not included*.

Study endpoints	SAVR n = 14097	TAVR n = 1194	p-value
Primary end point			
30-day mortality	258 (1.8%)	20 (1.7%)	p=0.700
Permanent stroke	151 (1%)	15 (1.3%)	p=0.553
30-day mortality and permanent stroke	409 (2.9%)	35 (2.9%)	p=1.000
Secondary end points			
Readmission for deep sternal wound infection	44 (0.3%)	0 (0%)	p=0.053
Readmission for valve dysfunction	10 (0.07%)	0 (0%)	p=0.357

Table 5.4: Primary and secondary study endpoints.

Compared to SAVR, the TAVR patients were typically older, there were more males than females in both groups, and the mean BMI was similar in both as shown in table 5.2. The mean implanted valve size was 27mm in SAVR and 23mm in TAVR as shown in table 5.2 and valve size details are shown in table 5.3. Tissue valve implantation was most common and transfemoral access most common in the TAVR group which probably reflects some bias in the registry.

Patient preoperative risk factors between SAVR and TAVR can be summarized in Supplementary table B. Patients in the TAVR group had more previous CVA's and MI's while a significant portion of SAVR patients had had previous cardiac surgery. The TAVR patients presented with more symptoms, but both SAVR and TAVR equally presented with higher numbers of patients with preserved ejection fractions (EF >60%).

The primary composite end points of 30-day all-cause mortality and permanent stroke showed now significant difference between the groups (Table 5.4)

The complications between SAVR and TAVR were reviewed, and the major differences identified (Table 5.5). Heart block requiring PPM insertion was significantly higher in the TAVR group, while new postoperative arrhythmia, particularly AF or flutter was common in SAVR. Re-intubation was higher in the SAVR group, while vascular complications including aortic dissection and acute limb ischemia were more prevalent in the TAVR group and statistically significant.

Complication	SAVR n - 14097	% of cohort	TAVR n = 1194	% of cohort	P-value
New arrhythmia	4625	32.81	187	15.66	p<0.0001
Heart block	421	2.99	89	7.45	p<0.0001
AF/Flutter	4000	28.37	61	5.11	p<0.0001
Bradyarrhythmia	185	1.31	33	2.74	p<0.0001
New ventricular tachycardia	234	1.66	10	0.84	p=0.0299
New renal insufficiency	645	4.58	31	2.59	p=0.0013
Permanent stroke	151	1.07	15	1.26	p=0.5428
Pulmonary embolism	20	0.14	0	0	p=0.1958
Coma > 24 hours	37	0.26	1	0.08	p=0.2280
New deep sternal wound infection (DSWI)	63	0.45	1	0.08	p=0.0580
Pneumonia	473	3.36	38	3.18	p=0.7398
Aortic dissection	6	0.04	4	0.34	p<0.0001
Septicaemia	135	0.96	2	0.17	p=0.0055
Anticoagulation complication	105	0.75	5	0.42	p=0.1966
Acute limb ischemia	5	0.03	5	0.42	p<0.0001
Re-intubation	252	1.78	6	0.50	p=0.0010
Multi system organ failure	142	1.01	7	0.59	p=0.1566
GIT complications	182	1.29	17	1.42	p=0.7034

Table 5.5: Postoperative complications recorded in SAVR and TAVR groups.

Following the application of all variables for this logistical regression for propensity score model there was only 22 subjects within each group that could be matched with a matched tolerance of 1.

Using logistical regression when all patient factors considered for all patients who had SAVR and TAVR, the only preoperative factors that had an impact on 30-day mortality was cerebrovascular disease, respiratory disease, preoperative dialysis, angina, and hypertension (table 5.6). Excluded variables are shown in table 5.7.

Comparison between SAVR and TAVR groups from the database was performed on the entire cohort as propensity score matching was not possible.

Preoperative Factor	**P-value**
Cerebrovascular disease	p=0.007
Respiratory disease	p=0.010
Preoperative dialysis	p=0.016
Angina	p=0.031
Hypertension	p=0.048

Table 5.6: Preoperative variables influencing 30-day mortality outcomes across SAVR and TAVR groups following propensity score matching.

Preoperative Factor	**P-value**
Gender	p=0.173
Aboriginal/Torres Strait Islander	p=0.360
Age	p=0.717
History of smoking	p=0.739
Current smoker	p=0.298
Diabetes	p=0.298
Hypercholesterolaemia	p=0.451
Peripheral vascular disease	p=0.622
Previous MI	p=0.116
Congestive cardiac failure	p=0.341
NYHA Class	p=0.944
PPM in-situ	p=0.273

Table 5.7: Excluded variables following propensity score matching amongst SAVR and TAVR groups.

5.7 Discussion

This study has identified significant differences between SAVR and TAVR clinical outcomes, namely that SAVR has greater prevalence of postoperative arrhythmias and re-intubation, whereas TAVR has increased heart block requiring PPM and vascular complications including aortic dissection and acute limb ischemia. These differences had not been highlighted or shown to be significant in previous RCTs comparing the two groups. Following propensity matching and identification of impactful preoperative factors, it is likely these included variables impact on post procedure recovery, particularly for patients who have been on cardiopulmonary bypass and whom have had a prolonged recovery period. This particularly is in reference to cerebrovascular disease, respiratory disease, and preoperative dialysis patients.

The most well-known clinical trials reporting on clinical outcomes in TAVR are the partner trials (Table 5.8) which report on outcomes in SAVR and TAVR patients in low, medium, and high-risk groups, and include prospectively selected patients that are likely to have better outcomes than the 'real world' data from registries.

Study	Year	Cohort	Outcomes
Mack et al (Partner 1) [5]	2015	High risk SAVR and TAVR (mean STS score 11.5%)	• The primary outcome of the trial was all cause mortality at 1 year, with secondary endpoints being stroke, readmission, AKI, vascular complications, and bleeding events. • Periprocedural stroke or TIA was higher in TAVR (5.5%) versus SAVR (2.4%), and transapical TAVR had higher mortality compared to transapical SAVR. • At 5 years there was no significant difference in all cause or cardiovascular mortality, stroke, or re-admission between SAVR and TAVR. • Moderate or severe aortic regurgitation caused by paravalvular regurgitation was more common in the TAVR group and was associated with lower survival. Author and investigator reasoning for the differences between paravalvular leak and clinical outcomes relate to valve development, operator expertise and experience, and patient selection for such trials.
Leon et al. (Partner 2) [9]	2016	Intermediate risk SAVR and TAVR (mean STS score of 5.8)	• The primary outcome of the trial was death of any cause or disabling stroke at 2 years, with secondary endpoints being vascular complications, life-threatening bleeding, AKI, new onset AF, re-admissions, PPM implantation, length of stay and paravalvular aortic regurgitation, in addition to others. • There was no significant difference in the primary end points of death and disabling stroke between SAVR and TAVR. • At 30 days, vascular complications were more frequent in TAVR (7.9%) versus SAVR (5%). Life-threatening bleeding was reported to have occurred more frequent in SAVR (43%) versus TAVR (10%), as well as new onset AF in SAVR (26%) versus TAVR (9%). The need for PPM was higher in TAVR (8.5%) than in SAVR (6.9%). • The frequency and severity of paravalvular aortic regurgitation was greater after TAVR (22.5% mild and 3.7% severe) versus SAVR. The severity of paravalvular leak worsened at 2 years in the TAVR group, and those who had moderate to severe regurgitation had higher mortality within 2 years. This was statistically significant with a p=value of <0.001). When explored in more detail, mild paravalvular leak worsened from 30 days to 2 years in the TAVR group in a reduced number of patients with supporting echocardiographic findings. • Moderate or severe paravalvular leak worsened from 30 days to 2 years in the TAVR group, while in the SAVR group; mild, moderate, or severe paravalvular leak improved over time. • Trial conclusions was that SAVR and TAVR outcomes in respect to death and disabling stroke are similar in intermediate-risk patients, and it was deemed that the TAVR

			expandable prosthesis may reduce patient prosthetic mismatch and result in greater long-term outcomes; and paravalvular leak resulted in increased mortality in the moderate to severe TAVR group.
Mack et al. (Partner 3) [10]	2019	Low risk SAVR and TAVR (mean STS 1.9%)	• The primary outcome was death, stroke or rehospitalization at 1 year; with secondary endpoints being new onset AF at 30 days, length of hospital stay, improvement in heart failure symptoms and functional outcomes as measured by a 6-minute walk test. • At 1 year, death from any cause was higher in the SAVR group (2.5%) versus the TAVR group (1%). Stroke was higher in the SAVR group (3.1%) versus the TAVR group (1.2%). Rehospitalization was higher in the SAVR group (11%) versus TAVR group (7.3%). • Heart failure symptoms (NYHA II, III, IV) were reported at 30 days and at 1 year. At 30 days symptoms were worse in SAVR (33.3% of patients versus 19.7%), but at 1-year symptoms were worse in TAVR at 17.7% of patients versus 16.7% in the SAVR group. • The percentage of mild paravalvular regurgitation at 1 year was higher in the TAVR group (29.4%) versus the SAVR group (2.1%). • Trial conclusions was that among patients with severe aortic stenosis who were at low risk for death with surgery, the rate of composite of death, stroke, or rehospitalization at 1 year was significantly lower with TAVR than with SAVR [7].

Table 5.8: Randomised control trial (RCT) Partner trials 1-3 comparing SAVR and TAVR outcomes in high intermediate and low risk groups.

Several other studies have reviewed the SAVR and TAVR outcomes in all risk groups (Table 5.9).

Study	Year	Cohort	Outcomes
Rosato et al. (Observant study) [11]	2016	Low risk SAVR vs TAVR	• Improved 3-year survival was better in SAVR (83.4%) versus TAVR (72%), and freedom from major cardiac and cerebrovascular events was greater in SAVR (80.9%) versus TAVR (67.3%).
Tyregod et al. (Notion study) [12]	2015	High risk SAVR vs TAVR	• They found no significant difference between SAVR and TAVR in the areas of composite death rate of any cause, stroke, or MI after 1 year.
Reardon et al. (Surtavi trial) [13]	2017	Intermediate risk SAVR vs TAVR	• The incidence of the primary end point (death or disabling stroke at 2 years) was 12.6% in the TAVR group and 14% in the SAVR group; with TAVR deemed a suitable non-inferior alternative to SAVR in this patient group.

Table 5.9: Prospective studies comparting SAVR and TAVR outcomes.

Several institutions have reported on their own registries (state based and national) comparing SAVR and TAVR outcomes in the evolving TAVR field (Table 5.10).

Study	Year	Cohort	Outcomes
Moat et al, Duncan et al. [14] [15]	2011	TAVR outcomes	• Reported a 92.9% 30-day survival in patients undergoing TAVR, 1-year survival was 78.6% and 2-year survival 73.7% (10) • Follow-up study on the same group of patients revealed a 3-year survival of 61% and 5-year survival of 45%, which was deemed respectable (11).
Eltchaninoff et al (France registry) [16]	2011	High risk SAVR and TAVR outcomes	• Reported a high predictive operative mortality (18.9%) and mean age of 82 years reported a 12.7% 30-day operative mortality and initial stroke rate of 3.7%.
Gilard et al. (France 2 trial) [17]	2012	High risk SAVR and TAVR outcomes	• Reported that 30-day operative mortality reduced to 9.7%- and 1-year mortality was 24% in a similar high risk, elderly cohort. The major stroke rate had decreased to 2.3%.
Grant et al. (United Kingdom registry follow-up) [18]	2016	SAVR and TAVR outcomes	• Observed a 30-day mortality of 2.1% in SAVR and 6.2% in TAVR as well as 5-year survival rates of 82% and 46% respectively.
Hamm et al, Mohr et al, Walther et al (German aortic valve registry) [6] [19] [20]	2017	SAVR and TAVR outcomes	• One-year follow up demonstrated excellent results in the SAVR group, and TAVR was deemed a good alternative for elderly and high-risk patients (15) (16). • Severe complications in TAVR patients have steadily decreased over time (17).
Thourani et al (Canadian registry) [21]	2017	High risk TAVR outcomes	• Evidenced relatively high mortality rates associated with TAVR in extreme-risk patients at mid-to long term follow up (24% at 1 year, 56% at 4 years).
Brennan et al (Transcatheter valve registry/STS database) [22]	2017	Intermediate and high risk SAVR vs. TAVR	• In both SAVR and TAVR, there was no significant difference in rates of death (17.9% versus 17.3%), and stroke (3.3% versus 4.2 %).
Barbanti et al (The Italian Observant study) [23]	2019	Low risk SAVR vs. TAVR	• At 5 years, the rate of death from any cause was 35.8% in SAVR and 48.3% in TAVR (p=0.002). In addition, TAVR was associated with increased risk of major adverse cardiac and cerebrovascular events (54%) versus SAVR (42.5%).
Virtanen et al (Finn Valve registry) [24]	2019	Low risk SAVR vs TAVR	• Mortality at 30-days was 3.6% in SAVR and 1.3% in TAVR. Three-year survival was 87.7% in SAVR and 85.7% in TAVR.

Table 5.10: National registries comparing SAVR and TAVR outcomes.

The most recent reviews of outcomes of SAVR and TAVR groups in Australia and worldwide including the Partner trial 5-year outcomes are shown in Table 5.11.

Study	Year	Cohort	Outcomes
Zweng et al [25]	2016	High risk SAVR and TAVR patients with propensity matched cohort	● Primary end points were 30-day mortality and 2-year survival with secondary endpoints looking at readmission within 30-days, new AF, heart block requiring PPM, significant paravalvular leak (>mild AR), stroke, pneumonia, and blood transfusion requirements (12). ● Survival at 2 years was 74% for TAVR and 80% in SAVR (in propensity matched pairs which yielded 44 pairs). ● In the propensity matched analysis, 30-day mortality was 5% in both groups, requirement of PPM was higher in TAVR at 23% and 5% in SAVR, postoperative AF was higher in SAVR at 41% and 2% in TAVR. ● The rates of paravalvular leak were 7% in TAVR and 0% in SAVR. Lack of statistical significance in the leak rate is likely due to lack of statistical power. ● TAVR patients included were of high operative risk and no validated frailty score was used to guide treatment allocation.
Makkar et al. [26]	2020	5-year outcomes for Partner 2 investigators of SAVR and TAVR patients Funded by Edwards Lifesciences	● At 5 years, death from any cause or disabling stroke was 47.9% in the TAVR group and 43.4% in the SAVR group. In the transfemoral access group, this was 44.5% and 42% respectively. In the transthoracic access group, this was 59.3% in the TAVR group and 48.3% in the SAVR group. In the overall population the incidence of death from any cause was 46% in the TAVR group and 42.1% in the SAVR group (56.9% in the transthoracic group for TAVR and 47.3% in the SAVR group). ● Rehospitalisation at 5 years was higher in TAVR with 33.3% versus 25.2% for SAVR. Aortic valve intervention was higher in TAVR with 3.2% versus 0.8% in SAVR (10/21 cases due to progressive stenosis and 11/21 due to worsening aortic regurgitation in the TAVR group). ● The postoperative aortic insufficiency was graded as mild or greater in comparing both groups. In independent analysis, at 5 years mild paravalvular leak was seen in 17% of TAVR patients and 3.5% of SAVR patients. At 5 years, moderate or severe paravalvular leak was seen in 4.1% of TAVR patients and 0.2% of SAVR patients. ● The main findings were that there was no significant difference in primary end points of death from any cause or disabling stroke at 5 years; but TAVR was associated with higher incidences of mild, moderate, and severe paravalvular regurgitation, and valve related intervention and rehospitalization was higher in the TAVR group versus SAVR.

Table 5.11 : The most recent review of SAVR and TAVR outcomes including the Partner Trial randomized control trials 5-year outcomes.

The Partner results may not replicate real world outcomes for several limitations that exist when performing a highly controlled RCT that otherwise do not exist in large scale registries, such as a collection of broad data over an extended period, highly controlled inclusion, and exclusion criteria, and outcomes bias. The ANZSCTS database has greater than 95% data completeness for all reported key performance indicators (KPI's) including in-hospital and, 30-day mortality; re-operation for bleeding; new renal insufficiency; deep sternal wound infection, and permanent stroke. Other performance indicators include new cardiac arrhythmias; duration of intensive care unit stay; duration of ventilation; and red blood and non-red blood cell transfusions. The aim of the database is to maintain a high standard of care for Australian and New Zealand cardiac surgery patients, and this is achieved through peer review of unit performance on a quarterly basis, and the feedback of performance information to sites.

Albeit the two groups have a very different subset of patients (with SAVR patients being much younger at baseline, and with a high amount of re-do cardiac surgery) preoperative risk factors such as HTN, previous CVA, respiratory disease or previous MI were similar. Preoperative LVEF was also similar between groups, with most patients (>50%) having a normal LV function (>60%) despite a range in NYHA symptoms. The differences between the two groups are highlighted in the statistical analysis (Table 5.1).

Primary end points showed no significant difference between patient groups, supporting recent registry trial results [5-7] [9] [10] [12] [13] [19] [20] [22]. Secondary end points, including the rate of readmission for valvular dysfunction was low in both groups (0.07% in SAVR and 0% in TAVR) and showed no difference ($p=0.3573$); suggesting that over this period, the degree of aortic insufficiency is not manifesting clinically. This is also supported in the Partner trials [5] [9] [10].

Additional secondary end point of readmission for infection showed no difference between groups ($p=0.0532$), and was not significant in the trials listed above. The rate of paravalvular leak postoperatively has been higher in TAVR groups throughout the analysis of SAVR and TAVR outcomes [5] [9] [10] [25] [26]. This data is not captured in either group in this database analysis unfortunately.

The SAVR results of increased re-intubation prevalence is both supported [27], and reported as showing no difference between groups [28]. Acute limb ischemia was also significantly higher in the TAVR group, and this would be influenced by the route of access chosen. Given most cases are transfemoral then this risk is understood [29], but incidence also depends on the technique used to access the femoral vessels. Acute dissection was also higher in the TAVR group and statistically

significant, which carries an incidence of 0.6-1.9% [30], and is thought to result from stiff wire interaction in the ascending aorta, catheter valve injury to the aortic wall by creating an intimal disruption, valve retraction to expose the balloon in balloon-expandable systems, balloon valvuloplasty injury, or post dilatation balloon interaction with the aorta [31].

New postoperative arrhythmia including AF and flutter was higher in SAVR and statistically significant, and occurs in up to 65% of patients undergoing open cardiac surgery, and is a known risk factor for mortality [32]. Replacement of the aortic valve can result in conduction defects due to the anatomical proximity of the AV node to the aortic annulus in both SAVR and TAVR groups [33]. Bradyarrhythmia's, VT episodes, and CHB was higher in TAVR; showing statistical significance in bradyarrhythmia's and new heart block, and has a similar causative factor being the proximity of the conduction pathway, with LBBB occurring in up to 70% of TAVR cases [33]. The rate of complete heart block (CHB) remains high in the TAVR group over this long-term analysis. Albeit CHB has been reported to be as high as 33% [25], the rate in this analysis was 7.45% in TAVR compared to 2.99% in SAVR, and this was statistically significant ($p<0.0001$). It was reported in a meta-analysis in 2014 [34] and supported in 2017 [25], that while PPM post procedure was needed, this PPM implantation had no negative impact on patient's survival despite increasing costs. Patients with CHB are vulnerable to decreased perfusion related to symptomatic bradycardia and decreased cardiac output, syncope related falls and head injuries. Other complications of treatment for CHB include pacemaker lead dislodgement, cardiac perforation, and pacemaker associated heart failure in the long term [35] [36].

Comparison of this Australian experience to the large Northern American (PARTNER) and GARY experiences over the last 10 years is important because these international registries capture large patient numbers over a range of clinical risk profiles and subsequently observational data and trends cannot be understated.

Rates of death, stroke, vascular complications, need for pacemaker, and moderate or severe paravalvular regurgitation have declined significantly in the TAVR population over the past decade of PARTNER trials [37]. However, some definitions were ambiguous, of limited clinical utility or required updating/extension. For example, if an unplanned percutaneous or surgical procedure did not lead to an adverse outcome it was not considered a major vascular complication. The issue of subclinical and clinical valve thrombosis has been increasingly recognised, with a reported incidence between 7% and 14%, and given expansion of TAVR into low risk-patients, long-term valve durability becomes an issue. Durability will become clearer as we extend into 10-year durability results. In comparison to the Australian experience, there were declines in vascular complications, declines in paravalvular leaks and overall stable rates of new pacemakers post procedure.

Similar to the PARTNER trials [5] [9] [10], issue of valve durability remains open in the GARY registries. Patient cohorts recruited in 2018 and 2019 in younger age groups will focus on this long-term durability with echocardiography in the next 10 years. In comparison to the Australian experience and other RCTs including North America, SAVR cohorts have maintained a very low in hospital mortality (2.1%) compared to TAVR (5.1% in transvascular groups) [38]. Severe vital complications (death on the same day, conversion to sternotomy, low cardiac output that required mechanical supports, annular rupture, and aortic dissection) occurred in 5% and technical complications were registered at 4.7% in the initial 15,964 TAVR procedures from 2011 to 2014 [38], however in recent years in these registries have been resolved and align with North American data.

Due to a divide between ANZSCTS and Transcatheter Aortic Valve Implantation Registry (TAVI ACOR) databases in TAVR data throughout Australia, a limitation in this analysis was not capturing all TAVR cases submitted into the TAVI-ACOR registry; however, a recent analysis of TAVR cases obtained from the TAVI-ACOR registry in 2019 only showed 865 in hospital cases collected up to this period (less than our 1,194) [39]. Therefore, this analysis captured a significant proportion of all documented TAVR procedures over the period utilised in our data collection. Further limitations in the interpretation of the ANZSCTS national database is the short-term (30-day) postoperative data that is collected. From the trends in the literature since TAVR was introduced, real world information concerning its value and clinical application results from long term analysis of outcomes and complications which are starting to appear [26]. There have been multiple RCTs [5] [9] [10] and subsequent long-term studies [26] which reveal progressively worsening valvular incompetence, a rate of CHB and PPM insertion, and vascular complications, including aortic dissection that are higher in the TAVR group versus SAVR group, and these findings have been replicated in our database analysis.

It should be acknowledged that that preoperative demographics of patients in this observational study have different comorbidities which may influence the results. This is a limitation of any observational study and is likely due to and may be influenced by selection bias. We attempted to address this by using propensity score matching although we were unable to able to identify a sufficient number of matches to perform a meaningful analysis. Nevertheless, the difference in outcomes appear to be related more to the unique technical challenges associated with each procedure.

5.8 Conclusion

With an understanding of limitations in TAVR ANZSCTS data to date, this database analysis was deemed to have an insufficient number of participants for analysis and comparison between groups. Despite these recognized limitations, SAVR and TAVR outcomes over an 18-year period showed good primary endpoint results in mortality and permanent stroke across both groups, and readmission for surgical or valvular complications. Areas of difference remain in the degree of complete heart block and resulting need for PPM insertion, and vascular complications, including limb ischemia and dissection. Although primary and secondary end points have remained similar across the two groups, secondary complications are severe and life threatening, and have shown to be significant in this analysis. There would be value in a combined or linked ANZSCTS and ACOR-TAVI database to capture the outcomes of these complications and perform complex analyses that carry high morbidity and mortality in the short and long term.

5.9 References

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CHAPTER SIX

Quality of life and frailty outcomes following surgical and transcatheter aortic valve replacement

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6.1 Statement of authorship

Statement of Authorship

Title of Paper	Quality of life and frailty outcomes following surgical and transcatheter aortic valve replacement
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Contribution to the Paper	Primary investigator and author Questionnaire completion Publication formulation		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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6.2 Manuscript summary

Title

Quality of life and frailty outcomes following surgical and transcatheter aortic valve replacement

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6.3 Abstract

Background

Our objective was to report on the prospective outcomes in the areas of depression, quality of life, angina, and frailty in SAVR and TAVR patients with aortic stenosis undergoing aortic valve intervention.

Methods

We recruited 300 patients across 3 groups (TAVR, SAVR, and CABG) over 12 months. Depression, quality of life, frailty, and angina were assessed followed by propensity score matching.

Results

Using logistical regression when all patient factors considered for all patients who had SAVR and TAVR, the only preoperative factors that had an impact on 1-year mortality was hypertension and STS score. Quality of life improvements within each group over 12 months was significant (p-value =0.0001). Depression at 12 months between groups (p-value =0.0395) and within each group was significant (p-value = 0.0073 for SAVR and 0.0001 for TAVR). Angina was most frequent in TAVR

at 12 months in the QL (p=0.0001), PL (p=0.0007), and improvement was significant in the QL (SAVR p=0.0010, TAVR p= 0.0001) and PL (SAVR p=0.0002), TAVR p=0.0007) domains in both groups. Frailty at 12 months improved in both groups, but was greatest in TAVR (p-value = 0.00126).

Conclusions

This 12-month follow up of cardiac surgical patients has revealed significant improvement in PROMs and frailty in all groups by 3-months postoperative regardless of surgical or transcatheter approach. Outcome measures of quality of life and frailty should be utilized as a measure of outcome more regularly in patients undergoing aortic valve surgery regardless of approach.

6.4 Background

Aortic valve replacement is designed to prolong life and improve its quality, with the latter being particularly relevant given the elderly patient's undergoing this procedure. The early studies reporting on quality-of-life analysis in aortic valve surgery patients were first published in 1997 [1] [2] [3]. PROMS were first applied in the areas of heart failure [4] and later to heart valve surgery in 2016 [5]. And determined the value in assessing a patient's quality of life before and after cardiac surgery.

Our primary endpoint is to determine quality of life between SAVR and TAVR in aortic stenosis (including CABG as a control) over a 12-month period. Our secondary aims are to determine and compare the angina, depression, and frailty outcomes between these groups. We hope that this information will help guide preoperative, perioperative, and postoperative management of patients undergoing aortic valve replacement in these crucial domains that determine patient satisfaction post aortic valve intervention.

6.5 Methods

6.5.1 Patient recruitment

Following ethics and governance approval (CALHN) (HREC/18/CALHN/188), between June 2018 and August 2020, a total of 300 patients across 3 groups were recruited consecutively from a single institution, at the Royal Adelaide Hospital, Adelaide, South Australia. The 104 three groups comprised a SAVR (100 patients), TAVR (100 patients) and coronary artery bypass grafting (CABG) group (100 patients). All patients were contacted directly, and consent obtained to participate in this data collection that would occur over a 12- month period. Inclusion criteria was patients undergoing a single cardiac procedure (SAVR, TAVR, CABG only) without associated

coronary intervention (PCI). Patients excluded had combined procedures, a major perioperative complication precluding continued involvement, patients who died, or who declined involvement. Those patients who declined involvement were replaced with a newly recruited patient to reach the prespecified sample size.

6.5.2 Baseline demographics

Socio-demographic, symptoms, comorbidities, and risk factors were collected at baseline from the patients as well as hospital records as presented in Table 6.1.

Average baseline Characteristic	**SAVR n=100**	**TAVR n=100**	**CABG n=100**
Mean Age	65.94 (SD 11.6)	82.87 (SD 6.9)	65.90 (SD 10.0)
Gender (male)	79/100	80/100	79/100
Diabetes Mellitus	19/100	38/100	46/100
Hypertension	56/100	69/100	74/100
Previous Stroke/TIA	5/100	6/100	11/100
AF	10/100	32/100	6/100
eGFR <90 ml/min	1/100	26/100	8/100
Pulmonary HTN	2/100	2/100	0/100
COPD	13/100	12/100	14/100
Existing PPM	1/100	13/100	0/100
PVD	1/100	10/100	2/100
NYHA Class	2.23 (SD 0.7)	2.61 (SD 0.6)	1.13 (SD 0.4)
LVEF	57.95 (SD 8.4)	54.62 (SD 11.8)	54.33 (SD 11.0)
AVA cm2	0.99 (SD 0.5)	0.82 (SD 0.3)	2.67 (SD 0.7)
Mean AV gradient	46.57 (SD 15.4)	41.10 (SD 13.8)	5.71 (SD 4.9)
History of CAD	11/100	49/100	100/100
STS score (%)	1.18 (SD 0.4)	4.82 (SD 3.0)	0.77 (SD 0.4)

Cohort mortality	2/100	7/100	0/100

Table 6.1: Baseline demographics, comorbidities, and cardiac function obtained from the study cohort.

**TIA (transient ischemic attack), eGFR (estimated glomerular filtration rate), HTN (hypertension), COPD (chronic obstructive pulmonary disease), PVD (peripheral vascular disease), NYHA (New York Heart Association), LVEF (left ventricular ejection fraction), AVA (aortic valve area), CAD (coronary artery disease), STS (society of thoracic surgeons)*

6.5.3 Health Status Instruments

Depression was measured using the Patient health questionnaire 9 (PHQ-9) [5, 6, 7, 8, 9]. There are 9 domains in the questionnaire with a score assigned 0-3 (0 being no depressive thoughts and 3 being depressive thoughts nearly every day). A range of scores from 0-27 are possible. Scores of 5, 10, 15, and 20 represent cut points for mild, moderate, moderately severe, and severe depression, respectively [10].

Quality of life was measured using the Euro QOL EQ-5D questionnaire [5, 11, 12, 13, 14, 15, 16]. Quality of life scores were separated into 5 domains with a score of 1-3 giving the patient health profile [17]. A health state score of 1 indicates no problems, a score of 2 indicates some problems, and a score of 3 indicates extreme problems.

Frailty was measured using the Essential Frailty Toolset (EFT) which is a 4-item screening tool incorporating a chair rise activity which is self-reported, any cognitive decline which is reporter assessed, haemoglobin level, and serum albumin level. A score of 3 points indicates frailty [18] [19], while a higher score of >4 was associated with a reduced 2-year survival [19], and others associated higher all-cause mortality at 1,2, and 3 years with higher modified EFT scores [20].

Angina was measured using the Seattle Angina Questionnaire (SAQ-7). The SAQ7 consists of 7 questions that reports on activities performed over a 4-week period and any specific limitations or symptoms of angina that have impacted on the patient in this time. A score 128 of 0-35 is assigned with 0 indicating the most limitation, pain, and impact on the patient's quality of life. Three domain scores and one summary score are generated from the SAQ-7 [21].

- A Physical limitation score (SAQ7-PL). The Physical limitation score assesses the degree of physical limitation over the past 4 weeks due to various activities representing mild, moderate, and severe exertion.

- An Angina frequency score (SAQ7-AF). The Angina frequency score assesses the frequency of angina symptoms over the past 4 weeks with higher scores representing lesser angina burden.

- A Quality-of-life score (SAQ7-QL). The Quality-of-life score assesses how the patient perceives their CAD to be impacting his or her QOL.

- A SAQ7 summary score. The SAQ summary score assesses the average of SAQ-PL, SAQ-AF, and SAQ QL scores [21].

6.5.4 Data collection

Questionnaire data was collected at five independent time periods as inpatient or by telephone questionnaire during the 12 months. The time periods consecutively collected were preoperatively (within 4 weeks of procedure), postoperatively (prior to hospital discharge), 3 months postoperatively, 6 months postoperatively, and 12 months postoperatively.

Data was collected by two investigators over this period, with each investigator reviewing the questioning process and data collection to ensure interobserver reliability. Data analysis was completed by the primary investigator.

6.5.5 Statistical analysis

Power for recruitment sample size was calculation at 0.05 and 90% power accounting for a 10% dropout rate with 110 patients recruited to satisfy power. Statistical analysis was performed using GraphPad Prism 6 (GraphPad So 152 software, San Diego, California). A P-value of <0.05 was considered significant.

An unequal variance t-test (Welch's t test) was used to compare SAVR and TAVR EQ5D health state, and 12-month EQ5D outcomes due to their equal means and normal distribution. A non-parametric test (Mann-Whitney U test) was used to compare EQ5D health score preop and at 12 months in SAVR and in TAVR due to differences in median and not-normally distributed independent groups. It was used to compare SAQ7 preoperative and 12-month scores between SAVR and TAVR, compare preoperative and 12-month scores in the SAVR group and independently in the TAVR group. This was performed in all subdomains of the SAQ7 test. It was used to compare preoperative and 12-month PHQ9 scores between SAVR and TAVR, preop and 12-month scores in the SAVR group and independently in the TAVR group. It was used to compare preoperative and 12-month EFT scores between SAVR and TAVR, and compare preoperative and 12-month scores in the SAVR group and independently in the TAVR group.

Logistical regression followed by propensity score matching was performed using SPSS. We performed a stepwise logistical regression analysis using all known patient preoperative demographics and co-morbidities that were collected. The dependent variable was 1-year mortality. Propensity matching was subsequently performed using the outcome of the logistical regression analysis with a tolerance of up to 1.

6.6 Results

A total of 331 patients were approached during the study to participate in the data collection process. A total of 31 patients declined to be involved for various reasons and subsequently were not included in the data analysis. No patients during the 12-month period declined to continue their involvement in the study, and no patient was lost to follow-up, however 9 patients died through the 12-month data collection period: 7 patients from the TAVR group and 2 patients from the SAVR group.

6.6.1 EQ-5D depression measurements

SAVR had the best quality of life regarding mobility (1.10) followed by TAVR and CABG respectively, p=0.40. In terms of self-care, CABG had the best quality of life (1.01), followed by SAVR and TAVR, p=0.40. In usual activities, CABG had the best quality of life (1.57) followed closely by SAVR (1.59) and TAVR, p=0.02 and 0.42 respectively. Pain and discomfort were best in the TAVR group (1.24) followed by SAVR and CABG, p=0.04 and 0.30. In terms of anxiety and depression symptoms, TAVR reported least symptoms (1.07), followed by CABG and SAVR, p=0.02 and p=0.07. The EQ-5D testing domains are summarized in Table 6.2 and figure 6.1.

Domains	**Mobility**			**Self-care**			**Usual activities**			**Pain/Discomfort**			**Anxiety/Depression**		
	SAVR	TAVR	CABG	SAVR	TAVR	CABG	SAVR	TAVR	CABG	SAVR	TAVR	CABG	SAVR	TAVR	CABG
Preoperative	**1.07**	**1.46**	**1.44**	**1.26**	**1.85**	**1.36**	**1.64**	**2.00**	**1.75**	**1.45**	**1.26**	**1.67**	**1.29**	**1.17**	**1.14**
Postoperative	**1.19**	**1.17**	**1.35**	**1.37**	**1.24**	**1.29**	**1.7**	**2.10**	**1.87**	**1.51**	**1.54**	**1.56**	**1.37**	**1.06**	**1.20**
3-months	**1.08**	**1.03**	**1.05**	**1.2**	**1.13**	**1.02**	**1.59**	**1.81**	**1.49**	**1.34**	**1.26**	**1.23**	**1.17**	**1.00**	**1.01**
6-months	**1.11**	**1.03**	**1.04**	**1.1**	**1.18**	**1.01**	**1.55**	**1.73**	**1.39**	**1.27**	**1.12**	**1.23**	**1.04**	**1.01**	**1.01**
12-months	**1.07**	**1.03**	**1.04**	**1.11**	**1.25**	**1.01**	**1.47**	**1.66**	**1.36**	**1.24**	**1.04**	**1.19**	**1.10**	**1.00**	**1.01**

Table 6.2: Domain measurements of EQ5D Quality of life in the 3 cohorts over 12-month analysis period.

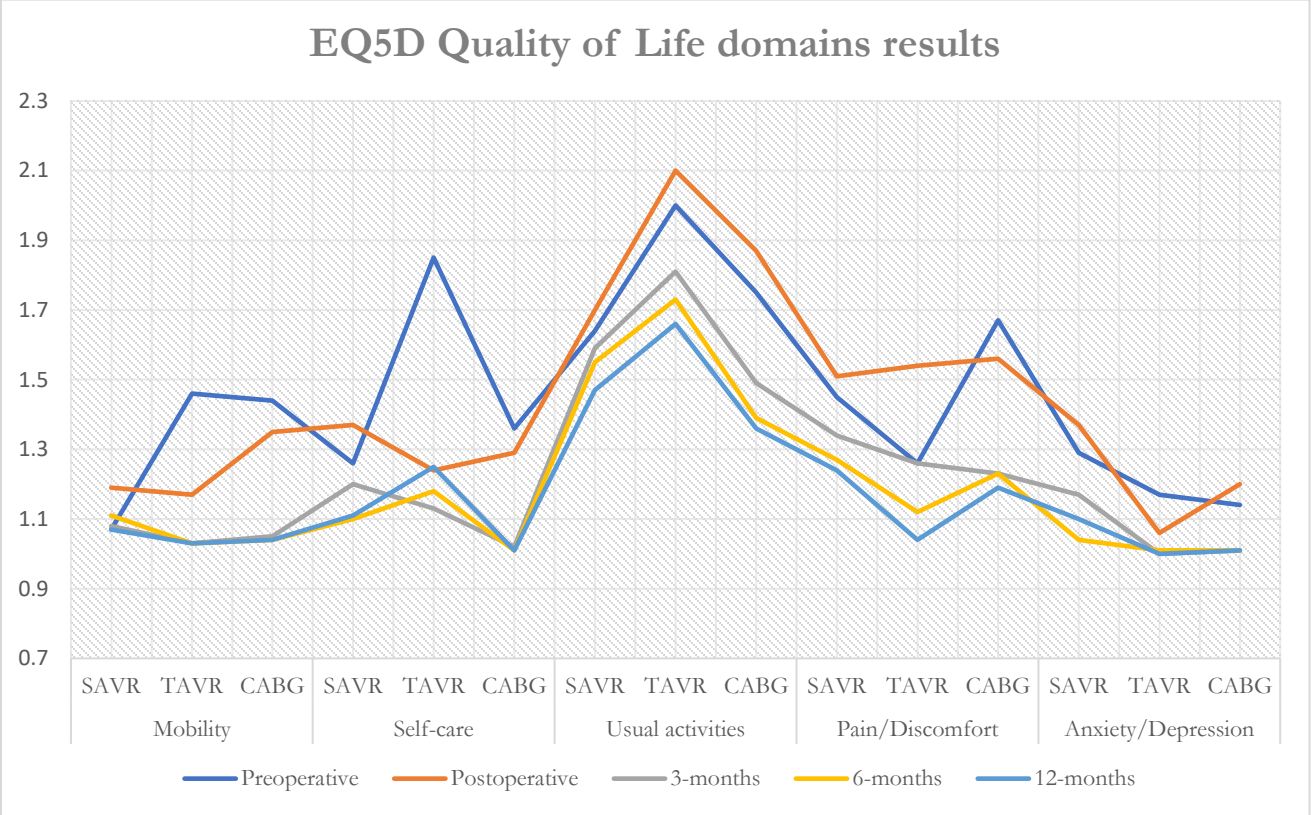

Figure 6.1: Line graph showing the distribution of QOL results within each domain amongst all groups.

Patient's own perspective of their health status over the 12-month period is summarized in Table 6.3. The best health status score was in the CABG group at 12-months, followed by TAVR and then SAVR.

Cohort	SAVR	TAVR	CABG
Preoperative	63.30	57.80	59.50
Postoperative	63.90	70.51	64.00
3- months	72.20	74.44	76.45
6- months	73.50	74.80	79.45
12-months	75.40	75.97	79.65
Average VAS score	**69.66**	**70.70**	**71.81**
Median	**72.2**	**74.4**	**76.5**
IQR	**63.6-74.5**	**64.2 – 75.4**	**61.8 – 79.6**

Table 6.3: Patient's own health score given over the 12 months period as a visual analogue scale (VAS) from 0-100. *A score of 100 indicates the best health a patient perceives themselves to be in at the time.

Patient results from their own perception of their health or the EQ5D Visual Analogue Scale (VAS) are shown in the Table 6.3 and figure 6.2.

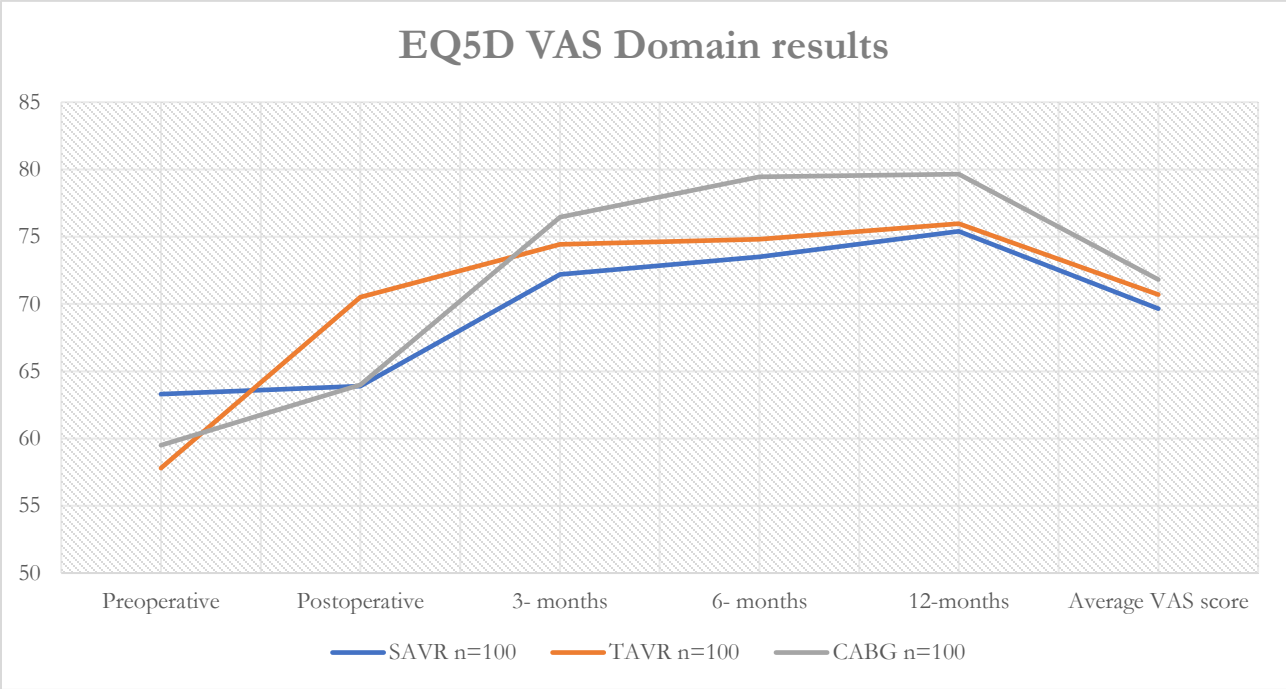

Figure 6.2: Line graph showing the distribution of QOL scores according to patients own health score as measured by VAS.

Each patient's preoperative and 12-month health state was determined in the SAVR and TAVR groups. Preoperative health state between SAVR and TAVR using an un-paired t-test with Welch's correction showed a significant difference (p-value =0.02). At 12 months, the SAVR and TAVR groups mean values were the same, and following statistical analysis as above, there was no significant difference between the two (p=value = 0.80). When comparing each group separately from preoperative to 12 months health state using the Mann-Whitney U test, SAVR showed a significant difference (p-value < 0.0001), and TAVR showed a significant difference (p-value < 0.0001).

6.6.2 PHQ-9 Depression measurements

Preoperative depression analysis using Mann-Whitney U test showed significant difference between SAVR (2.31) and TAVR (2.54) (p-value = 0.0142). SAVR (median 0.0, IQR 0 – 3); TAVR (median 2, IQR 0-4).

Postoperatively, the range was 0-13 in the CABG group, 0-13 in the TAVR group, and 0-16 in the SAVR group. At 3-month follow-up, depression scores ranged from 0-14 in the CABG group, 0-5 in the TAVR group, and 0-16 in the SAVR group. At 6-month follow-up depression scores ranged from 0-10 in the CABG group, 0-6 in the TAVR group and 0-15 in the SAVR group. At 12 months, depression scores ranged from 0 10 in the CABG group, 0-6 in the TAVR group, and 0-15 in the SAVR group. Postoperative depression analysis using Mann-Whitney U test showed significant difference between SAVR and TAVR (p-value = 0.03).

No patients reported symptoms of suicidal or homicidal ideation throughout the questionnaire process. Those who scored higher on the symptom scoring, were referred accordingly. Average depression scores were low in all groups. The SAVR group had the lowest score (1.51) followed by TAVR (1.56) and CABG (1.74) respectively.

Intergroup analysis of preoperative and 12-month depression scores using Mann-Whitney U test showed statistically significant results in the SAVR (p-value = 0.01) and TAVR (p-value =0.0001). Depression measurements as per the PHQ-9 questionnaire over the 12-month data collection period can be summarized in Table 6.4 and figure 6.3.

Cohort	**SAVR n= 100**	**TAVR n = 100**	**CABG n = 100**
Pre-operative	2.31	2.54	2.33
Postoperative	2.24	2.17	3.15
3-months	1.23	1.17	1.52
6-months	0.99	1.02	0.83
12 months	0.78	0.92	0.89
Average PHQ-9 score	**1.51**	**1.56**	**1.74**
Median PHQ-9 score	**1.23**	**1.17**	**1.52**

IQR	**1.0-2.2**	**1.0-2.2**	**0.9-2.3**

Table 6.4: PHQ-9 measure of depression over a 12-month period across the 3 cohorts. * A score of < 1 denotes no depressive symptoms and <5 minimal depressive symptoms

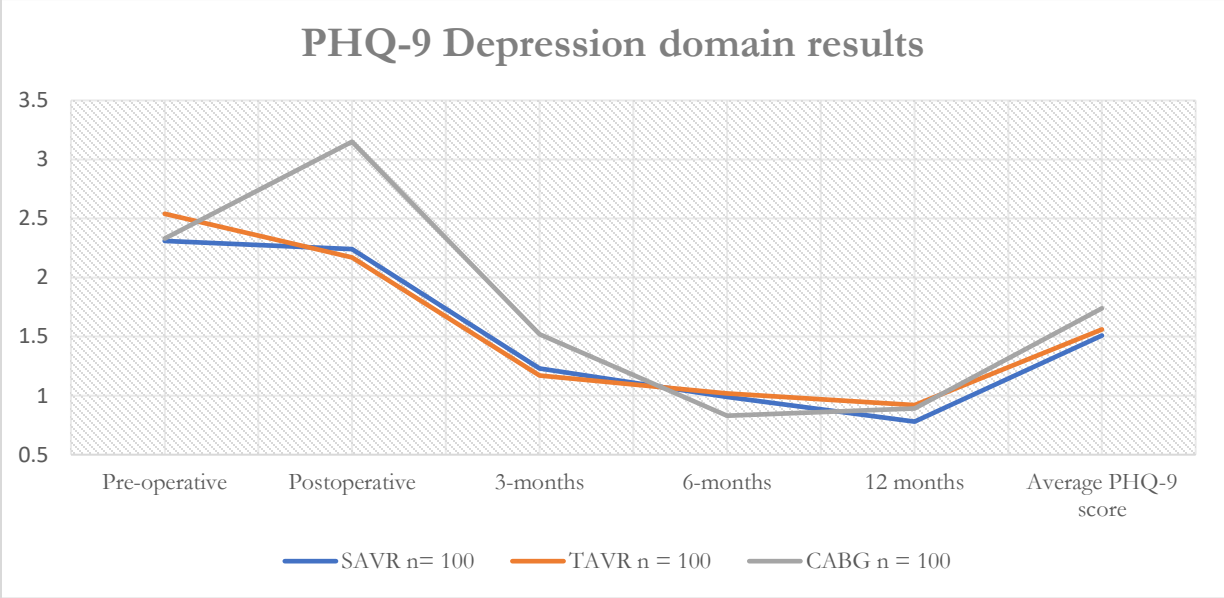

Figure 6.3: Line graph showing the distribution of depression scores over 12 months across all groups.

6.6.3 EFT Frailty measurements

Frailty in the TAVR group was worse preoperatively compared to SAVR. Using the Mann-Whitney U test, this was significantly different (p-value = 0.02).

Average frailty scores were higher in the TAVR group (0.98), and CABG group (0.97) compared to the SAVR group (0.83). Noticeably preoperative TAVR frailty scores were higher than the other cohorts (1.08). Only the CABG group in the postoperative measurements (3.15) reached a level of classification as frail. Statistically, the SAVR and TAVR differences at 12 months were not significant (p-value = 0.07).

Intergroup analysis revealed no significant difference 225 in frailty over the 12 months in the SAVR group (p226 value = 0.05) and a significant difference in the TAVR group (p-value = 0.01). Frailty measurements as per the EFT over the 12-month data collection period are summarized in table 6.5 and figure 6.4.

Cohort	SAVR n = 100	TAVR n = 100	CABG n = 100
Pre-operative	0.85	1.08	0.89
Postoperative	0.91	1.14	1.57
3-months	0.94	0.95	1.01
6-months	0.83	0.95	0.82
12-months	0.61	0.80	0.55
Average EFT score	0.83	0.98	0.97
Median EFT score	0.85	0.95	0.89
IQR	0.8-0.9	1.0-1.1	0.8-1.0

Table 6.5: EFT measurements of frailty over a 12-month period. Scores of 3 or > were classified as frail.

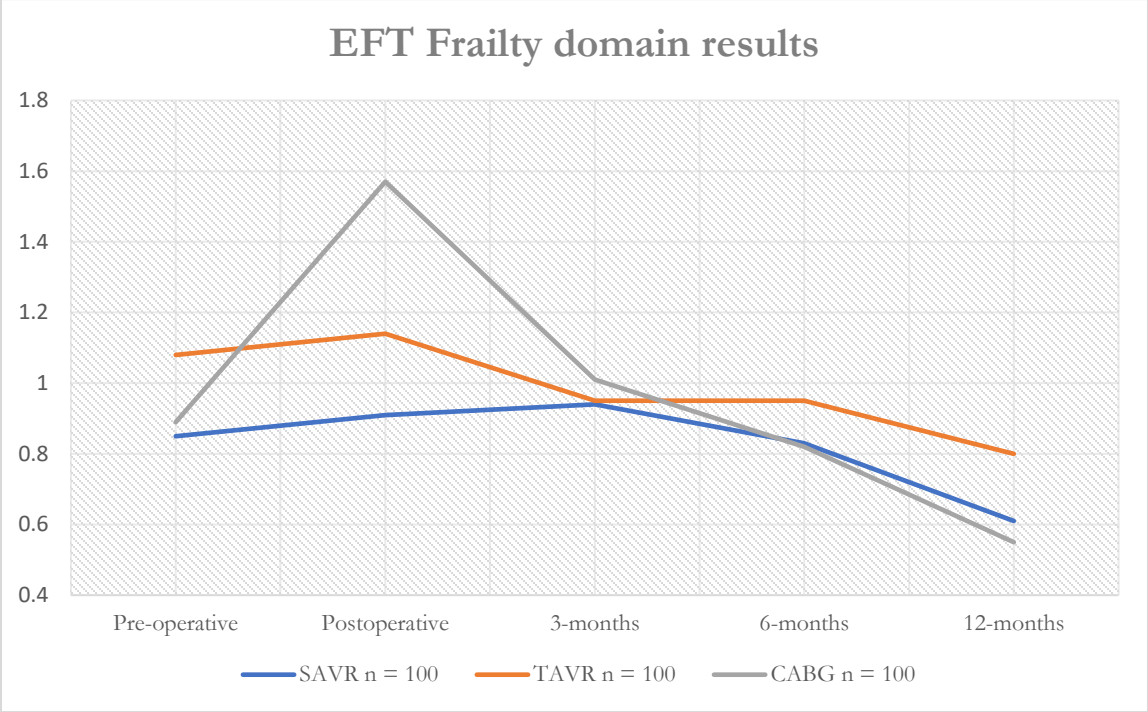

Figure 6.4: Line graph showing the distribution of frailty scores over 12 months across all groups.

6.6.4 SAQ-7 Angina measurements

In the measurement of angina outcomes, preoperative scores in the physical limitation (SAQPL) were worse in the CABG group (88.13), followed by TAVR (91.53) and SAVR (94.87) respectively. The difference between SAVR and TAVR preoperatively was significantly different (p-value = 0.0002). Scores in the angina frequency (SAQAF) were worse in the CABG group (84.66), and

almost equal in the SAVR (99.58) and TAVR (99.91) groups. The difference between SAVR and TAVR preoperatively was not significantly different (p-value = 0.1213). Quality of life (SAQQL) was equal preoperatively between CABG (90.50) and TAVR (90.60) and lower in the SAVR group (94.50). The difference between SAVR and TAVR preoperatively was significantly different (p-value < 0.0001). Summary scores across all subdomains indicated a higher angina score in the CABG group (87.76), followed by TAVR (94.01) and SAVR (96.32) respectively. The difference between SAVR and TAVR preoperatively was significantly different (p-value = 0.0001).

Postoperative scores in the SAQPL group were worse in the TAVR group (91.80), followed by CABG (92.93) and SAVR (94.53). SAQAF scores were higher in the CABG group (90.42), with almost equal scores in the SAVR (99.58) and TAVR (99.91) groups. SAQQL scores were higher in the TAVR group (91.50), with equal scores in the CABG (94.00) and SAVR group (94.10). Postoperative summary score showed higher CABG scores (92.45), followed by TAVR (94.40) and SAVR (96.07).

Scores obtained at 3-months postoperatively in the SAQPL domain showed higher CABG scores (94.80), followed by TAVR (95.47) and SAVR (96.87). Scores in the SAQAF domain showed higher angina scores in the CABG group (95.75) and no reported anginal frequency in both the SAVR (100) and TAVR (100) groups. SAQQL scores were highest in the TAVR group (95.00), followed by almost equal scores in the CABG group (96.10) and SAVR groups (96.20). Summary scores showed higher scores in CABG (95.55) compared to TAVR (96.82) and SAVR (97.90).

Scores obtained at 6 months postoperatively in the SAQPL domain showed higher scores in the TAVR group (96.00), followed by the CABG group (97.33) and SAVR group (98.00). Scores in the SAQAF domain showed higher scores in the CABG group (97.83) with no reported anginal frequency at 6 months in the SAVR (100) and TAVR (100) groups. Scores in the SAQQL domain showed highest scores in the TAVR group (95.30), followed by SAVR (97.6) and CABG (98.30). Summary scores were highest in the TAVR group (97.10) followed by CABG (97.82) and SAVR (98.53).

Scores obtained at 12 months postoperatively in the SAQPL domain showed higher scores in the TAVR group (94.67), followed by CABG (97.33) and SAVR (98.40). The difference between SAVR and TAVR was significantly different (p-value = 0.0007). Scores in the SAQAF domain were highest in the CABG group (97.83), followed by TAVR (99.58) and SAVR (100.00). The SAVR and TAVR 12-month scores were significantly different (p-value = 0.0251). Scores in the SAQQL domain were highest in the TAVR group (95.80) followed by SAVR (98.10) and CABG (98.30). The 12-month SAQQL scores were significantly different between SAVR and TAVR groups (p-value = 0.0001). Summary scores showed higher values in the TAVR group (96.68) followed by CABG (97.82) and SAVR (98.83).

Intergroup analysis showed a significant difference in the preoperative and 12 months SAQPL score in the SAVR group (p-value = 0.0002) and TAVR group (p-value = 0.0007). Intergroup analysis did not show a significant difference in SAQAF scores in the SAVR group (p-value = 0.1213) but was significant in the TAVR group after 12 months (p-value = 0.0251). Intergroup analysis showed a significant different in the SAQQL score for SAVR (p-value = 0.0010) and TAVR (p-value = <0.0001).

Scoring of the subdomains in the SAQ7 questionnaire over the 12-month analysis period can be summarised in Table 6.6 and Figure 6.5.

Score	**Preoperative**			**Postoperative**			**3 months**			**6 months**			**12 months**		
	SAVR	TAVR	CABG	SAVR	TAVR	CABG	SAVR	TAVR	CABG	SAVR	TAVR	CABG	SAVR	TAVR	CABG
SAQ-PL	94.87	91.53	88.13	94.53	91.80	92.93	96.87	95.47	94.80	98.00	96.00	97.33	98.40	94.67	97.33
SAQ-AF	99.58	99.91	84.66	99.58	99.91	90.42	100.00	100.00	95.75	100.00	100.00	97.83	100.00	99.58	97.83
SAQ-QL	94.50	90.60	90.50	94.10	91.50	94.00	96.20	95.00	96.10	97.6	95.30	98.30	98.10	95.80	98.30
SAQ7 Summary score	**96.32**	**94.01**	**87.76**	**96.07**	**94.40**	**92.45**	**97.90**	**96.82**	**95.55**	**98.53**	**97.10**	**97.82**	**98.83**	**96.68**	**97.82**

Table 6.6: Summary of the domain scores in the SAQ7 questionnaire including the patient SAQ Health score over the 12-month study period.

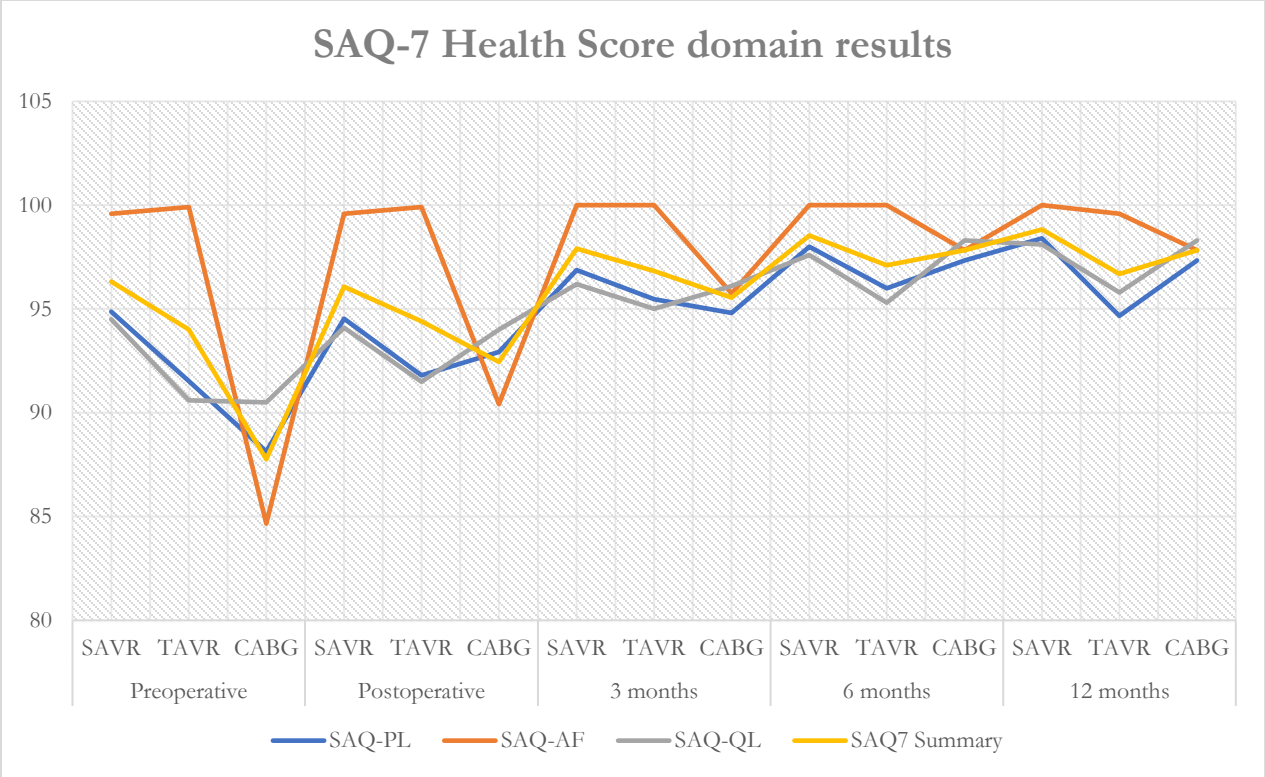

Figure 6.5: Line graph showing the domain scores in the SAQ7 questionnaire including the patient SAQ health score over the 12-month study period.

SAVR versus TAVR we matched a total of 58 patients across both groups. Using logistical regression when all patient factors considered for all patients who had SAVR and TAVR, the only preoperative factors that had an impact on 1-year mortality was hypertension, and STS score (table 6.7).

Preoperative Factor	**P-value**
HTN	p=0.0368
STS score	p=0.0040

Table 6.7: Preoperative variables influencing 1-year mortality outcomes across SAVR and TAVR groups following propensity score matching.

For the matched patients, we had a higher mean of 34.69 (SAVR) versus 34.07 (TAVR) for SAQ at 1-year which is statistically significant. The remaining results are not statistically significant but because of the low number of matched patients, a determination cannot be made (Table 6.8). Despite this, clinical significance of these outcomes and comparisons needs to be appreciated.

Questionnaire	Mean	P-value
SAQ7	SAVR (34.69). TAVR (34.07)	p=0.0429
PHQ9	SAVR (0.52), TAVR 0.63)	p=0.6978
EQ5D	SAVR (7.55). TAVR (7.66)	p=0.9530
EFT	SAVR (0.72), TAVR (0.96)	p=0.3100

Table 6.8: Statistical analysis following propensity matching between SAVR and TAVR in all questionnaires.

6.7 Discussion

In early registry data, [22] quality of life and frailty was extracted; however, the use of questionnaires was not included, including the PHQ-9, SAQ-7, and EFT. The Partner trials provided randomised outcomes between SAVR and TAVR [22, 23, 24], and reported that quality of life and health status were maintained at 12 months [23]. The Partner 2 trial in 2016 assessed baseline heath status using Kansas City Cardiomyopathy (KCCQ), SF 36, and EQ 5D questionnaires. This was reported over a 1-2-year follow-up [24]; and the Partner 3 trial in 2019 assessed functional status and quality of life at 30 days and 1 year using a 6-minute walk distance, and (KCCQ) score. Conclusions were that TAVR had rapid improvements in symptoms of failure, and 6-minute walk distance. [22]. Only the Partner 2 trial, used a specific quality of life questionnaire in the use of the EQ5D. In these large trials there has been less focus on quality of life and angina, and no reference towards depression and frailty as primary or secondary endpoints.

It should be identified that TAVR patients were much older with more medical comorbidities, compared to the SAVR and CABG control group.

When we summarise the collective findings of the study, we find that, quality of life outcomes was evenly distributed across the groups, depressive symptoms improved across all groups, and all groups including the TAVR group improved significantly in the measure of frailty at 12 months. Limited by power calculations and median similarities between median values, frailty results should be interpreted with caution.

Anginal scoring had the most complexity when it came to measurements of outcome. Compared to other instruments, the SAQ was the most responsive instrument to the anginal status and to the clinical change [25]. The SAQ was deemed more responsive than the SF-36 in terms of physical functioning when evaluating patients undergoing coronary bypass surgery (CABG) and angioplasty (PTCA) with a 3-month follow-up after revascularisation [26]. The improvement in physical limitation is noted in both SAVR and TAVR, while anginal improvement was highest in TAVR group compared to SAVR.

In comparing the SAVR and TAVR groups, both remained free of significant anginal symptoms throughout the preadmission and postoperative follow-up. The TAVR group started at a higher risk and older age group and despite this had a steady improvement in physical limitations, anginal frequency and quality of life over 12 months.

With the recognised importance in different presentations between men and women in ischemic heart disease [27–29]; measurements in quality of life could also be different in validated instruments. A retrospective multicentre analysis of over 10,000 patients including men and women showed comprehensive evidence that the SAQ is a valid patient-reported instrument that reliably helps capture the symptoms, functional status, and quality of life related to angina, while also providing useful prognostic information in women with CAD [17].

In terms of study limitations, this is a prospective cohort study and inherently contains a selection bias, minimised with data collected consecutively. This is supported by a reduced number of propensity matched patients, likely related to lack of power because of reduced patient numbers and therefore reduced statistically relevant conclusions. The EFT has only been validated in the preoperative setting. All PROMS were conducted over the phone and by two investigators, whereas frailty measures were determined through the collection of hospital data and over the phone in the measure of cognitive changes specifically. All three groups had different baselines and comorbidities. The data collection period was only over 12 months and will not capture the intermediate term complications. We recognise that the most important data will occur at least 7 years or more after a procedure when structural valve degeneration can have an impact. In an aim to reduce bias, propensity analysis via logistic regression analysis was performed.

Despite the limitations, the clinical value of such results should not be understated, and we hope could supply value to the outcome measures. The SAQ for example is well-established in its validity, reproducibility, prognostic importance, and sensitivity to clinical change, but interpretation can be challenging because of lack of familiarity with the clinical importance of its domains, either cross sectionally or longitudinally [30]. These questionnaires should be considered tools to support more patient-centred care, and a means of facilitating population health strategies to provide a better foundation for the integration of patient experiences with clinical care.

6.8 Conclusion

This study has shown that quality of life, depression, frailty, and angina improves across all groups of varied preoperative risk undergoing interventional and open cardiac surgical procedures over a 12-month period. Clinical evidence supports improvements across all domains and outcome measures for patients who undertake either SAVR or TAVR.

Following aortic valve surgery and coronary bypass surgery, symptoms impacting on a patient's quality of life reduce by 3 months postoperatively and improve to a point greater than their baseline functioning prior to their surgery regardless of pre-existing age and risk stratification. If we focus on optimising these areas, we may enhance a patient's perioperative quality of life when undergoing cardiac interventional and open surgical procedures.

6.9 References

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Chapter 7

Thesis conclusions

7.1 Major findings

This thesis has advanced the understanding, management, and how we should think about approaching disease affecting the aortic root, ascending aorta, and aortic valve.

The aortic root and ascending aorta length vary in length from approximately 7-15cm, and minor changes in the tissue and structural characteristics in this small region can have a profound effect on an individual's survival. Similarly, disease in these areas require surgical intervention, with medical therapy being suboptimal, and therefore how this small area is managed in surgery, further influences whether a person's outcome will be good or bad, and whether they will live or die. The distribution of the root and mid diameters in size groups, per se, brings into question their homogenized surgical management. Incremental knowledge on the vulnerability of the aortic root and ascending aorta measured clinically and histologically should not be underestimated. How these unique pathological characteristics are identified, appreciated, and managed could impact greatly on patient outcomes and overall survival.

There will always be an ongoing search for the least invasive ways to perform surgery, and this has emerged greatly in the realm of aortic valve replacement in recent years. However, less invasive does not always mean a better option from the patient's perspective. The application of TAVR in the management of aortic valve disease should be appreciated and utilised, but the less actively reported outcomes that affect a patient's clinical recovery need to be uncovered, and similarly aligned should be the emphasis and utilisation of patient related outcome measures that have similarly been advantageous in SAVR and TAVR. There is a focus on strict end points in the big, randomised trials, but this should not be at the expense of significant outcomes that will influence a patient and their family's quality of life. This thesis has helped to identify these.

7.2 The histological differences between the aneurysmal ascending aorta and aortic root (Chapter 2)

This chapter was a histological and immunohistochemical comparison of the microscopic structure of the aneurysmal aortic root and thoracic ascending aorta, of which direct comparisons in the literature are few It directly compared the core structural components of the aorta tissue (collagen

and elastin), allowed the determination of any structural difference that exists between these two regions, and if aneurysmal progression influence's structure.

Historically, ascending aorta pathology reported degradation of elastin, and collagen fibres [36, 66], whereas aortic root pathology has shown that the elastic fibre network breaks down and the connections with the complex collagen networks are lost. The collagen types change significantly; with collagen I and III decreasing and collagens XI and V increasing [28]. This study identified differences between the aortic root and ascending aorta collagen composition, and collagen types. Collagen content is increased in aortic root aneurysms compared to ascending aorta aneurysms, and is particularly increased in the areas of the sinus tissue, compared to the valvular regions. When comparisons are made between the aneurysmal aortic root and non-aneurysmal aorta, collagen content is higher, collagen I subtype is seen in more abundance, and elastin levels are reduced. Observational analysis supports these findings, with increased pathological changes occurring in the aneurysmal aortic root and proximal ascending aorta compared to other regions.

The aortic root and ascending aorta aneurysms are different in structure. Results show that the aortic root is a unique structure microscopically and histologically, and at an immunohistochemical level, and therefore supports its unique embryological and anatomical development. The safe conclusion is that the natural histories of the root and the ascending aorta are unique. These differences require further investigation and correlation to clinical outcomes.

7.3 The structural limits of the aortic root and ascending aorta in an ex-vivo porcine model (Chapter 3)

We aimed to identify any differences between the aortic root and ascending aorta in its biomechanical properties and structural limitations under stress. The known microstructural differences were used as a platform for demonstrating macrostructural differences. It has been progressively suggested that many ascending aorta patients who suffer a dissection do not have a markedly dilated aorta at the time [215] with 60% having diameters of <5.5cm and 40% having diameters of <5cm. The aim was to determine if the different areas of the thoracic aorta were influenced differently under high aortic stresses.

This was one of the few studies testing the structural limits of the intact thoracic aorta using a pressure testing apparatus, with previous studies focusing on aortic valve function, radiological analysis, and artificial replications of the aorta tubing using a balloon and reservoir (216). This study replicated high intraluminal pressures in the aortic root and ascending aorta lumen to determine its structural limitations.

The aortic root shows susceptibility to tearing and tissue failure at high luminal pressures compared to the ascending aorta, and the site of tearing was reproduced in all experiments. Macroscopic properties in this ex-vivo experiment supported that the aortic root is weaker than the ascending aorta and will fail at lower aortic pressures in the acute setting. This identified a new feature of the thoracic ascending aorta other than size that could predispose to failure in aneurysms.

7.4 The structural limits of the aortic root and ascending aorta in a live porcine model (Chapter 4)

The aim was to create a dynamic physiologically comparable experiment to support the findings of the aortic root having greater susceptibility to failure compared to the ascending aorta during high flow stresses. Furthermore, the aim was to utilise MRI imaging of wall sheer stress to determine if it is a contributing factor to identifying the areas of greatest stress during high aortic pressures.

By placing live pigs under cardiopulmonary bypass and pressurising the clamped aorta we were able to determine the weakest region of the aortic root and ascending aorta under stress. This study determined that the aortic root ruptured at the area of the non-coronary cusp in all experiments and this area was deemed the most susceptible to failure.

In addition to the observational analysis of the aortic rupture, histological analysis was performed to determine the changes that occur following immediate stress and aortic tissue failure. There was no significant increase in collagen content in the aortic root versus ascending aorta in this acute study, however collagen I was seen in greater content within the root versus the ascending aorta. This suggests that when comparing the acute pig aorta immunohistochemistry changes with the chronic aneurysmal aorta immunohistochemistry changes, collagen content is altered significantly within the aortic root, and that human and pig aortic analysis is comparable. Wall sheer stress via MRI imaging at high luminal pressures did not correlate to the area of rupture in our study.

This research clearly identified that the aortic root is the most susceptible region to rupture and failure at high pressures, and this supports previous ex vivo and histological studies in the earlier chapters. This study provided evidence of the area of greatest weakness between the root and ascending aorta, and hopefully larger scale studies using cardiopulmonary bypass will support these findings.

Chapter 2, 3, and 4 show collective evidence that there is a microscopic and macroscopic difference between the aortic root and ascending aorta, and this difference may be a contributing factor that predisposes the root to earlier aneurysmal progression and eventual rupture.

7.5 Clinical outcomes following SAVR and TAVR utilizing the ANZSCTS surgical database (Chapter 5)

At the most proximal level of the thoracic aorta within the aortic root, outcomes were determined in the management of disease affecting the AV, and how the two different ways of replacing this valve influences clinical outcomes.

With the enhancing transcatheter approaches to AV replacement, ANZSCTS database outcomes comparing SAVR and TAVR were determined. Previous ANZSCTS database analysis had reported on outcomes in a smaller cohort and over a shorter period with a focus on a single centre study. This research showed results over the last 18 years comparing outcomes to some of the larger Partner trial registries published in the last 5 years. There is a pre-determined focus on mortality and stroke outcomes, yet the effect of surgical morbidity cannot be understated, and therefore this was explored in detail.

Limitations existed in the groups tested, as TAVR patients were clearly older. However, many associated co-morbidities were similar. We could not capture an equivalent amount of TAVR patients due to procedure numbers, but also due to splitting of the TAVR registry between ACOR and ANZSCTS databases.

Mortality and permanent stroke remained as reported in previous RCT's, with no significant difference between the groups and in low percentages. What emerged from the secondary end point complications was a significant increase in heart block, bradyarrhythmia's, aortic dissection, and acute limb ischemia within the TAVR group, which have not been an area of great focus in previous large centre studies. These results should highlight the need to focus future studies on the implication of secondary end point complications on these two groups, and a combined AVR database may help achieve these registry goals with the greatest accuracy.

We have identified using logistical regression analysis the following variables (cerebrovascular disease, respiratory disease, preoperative dialysis, angina, and hypertension) to be predictive of early mortality. Optimisation of these conditions may lead to a reduction in early complications.

7.6 Frailty and Quality of life outcomes following SAVR and TAVR (Chapter 6)

In addition to the underreported morbidity post AV surgery noted in the previous chapter, frailty, and quality of life post SAVR and TAVR is an emerging yet scarcely reported topic. Quality of life outcomes were only reported in the Partner 2 trial (145). Smaller studies have reported on components of PROMS, but capturing depression, angina, quality of life, and frailty in a single study is a gap in the literature that could be filled in this research.

As reported in the previous chapter, there were differences in the baseline characteristics between the two groups, with TAVR patients being older, as well as having more co-morbidities and higher preoperative mortality risk scoring. Patient recruitment numbers were equal. Both groups showed significant improvement in quality of life, depression, and angina over 12 months following their surgery, and their perception of improved health status was also improved in both groups. This was also seen in frailty outcomes at 12 months, but it can be appreciated that the TAVR group (with increased age and comorbidities at baseline) showed the greatest improvement.

Although this study is limited by its use of telephone questionnaires and the measure of frailty limited by the power of patient recruitment, it provides results that support ongoing improvement in both validated questionnaires and patient health perceptions that correlate well with their clinical outcomes. The utilisation of perioperative questionnaires and frailty measures would have significant value in the assessment of outcomes for patients undergoing AVR in the future, and would aim to bridge a gap in an area that is underreported but so valuable to patient outcomes.

We have identified using logistical regression analysis the following variables (STS score and hypertension) to be predictive of early mortality. In a propensity matched cohort, the SAVR identified higher SAQ outcomes at 1 year suggestive of better angina outcomes. There was a non-significant trend that patients in the SAVR cohort in the propensity matched group performed better.

7.7 Limitations

With regards to the histological analysis of the aortic root and ascending aorta, the major limitations of this work were the variability of analysis between colour deconvolution measurements, and the results produced. Although performing each analysis twice enabled for improved accuracy amongst final measurements, the deconvolution process is susceptible to high variability. However, the gross percentages produced are less important to the overall analysis, and more importantly is the trend in results and comparisons between the major regional areas of the aorta.

With regards to the studies testing the functional limits of the aortic root and ascending aorta, the major limitations of this work were the static nature of the experimental design, which may not accurately represent the flows and pressure changes that occur in normal ascending aorta. Although the static fluid pressure testing was still a valuable comparison between the aortic regions, it may not accurately represent the flows and forces that would be experienced in cardiac physiology. The use of pig healthy aortas allowed the experiment to maintain the tubular aortic structure for testing and application to the apparatus, but limited its true comparison to the weaker aneurysmal aortic root and ascending aorta.

With regards to the testing of the limits of the ascending aorta and aortic root using a live functional pig model, one major limitation was the use of healthy aorta's instead of pathological aneurysmal aortas. This does allow direct correlation to the ex vivo functional study but limits our predictions on the effects in a pathological specimen. A study incorporating aneurysmal ascending aorta and aortic root pigs would not be possible with the resources and time that this study allowed, and would likely rely on artificial disruption of the aortic wall layers to create a pseudoaneurysm. This again, would not be a true representation of the natural development of an aneurysm. A further limitation would be the sample size utilised, however live animal surgical testing utilising cardiopulmonary bypass in large animals requires significant resources, and again this study was completed within the confines of available funding, in addition to literature driven power calculations. This study was only able to comment briefly on the impact of functional stresses using MRI 4D flow imaging comparing the root and ascending aorta, as well as histological analysis reporting on acute changes instead of chronic aorta changes.

With regards to the ANZSCTS database review on SAVR and TAVR outcomes, the major limitation in this work were the effects of observational analysis and by selection bias. We attempted to address this by using propensity score matching although we were unable to able to identify enough matches to perform a meaningful analysis. This database review focused on clinical outcomes over an extended period and involved large amounts of data and analysis.

With regards to the determination of clinical outcomes in SAVR and TAVR patients through frailty, and PROMS questionnaires, the major limitation was the use of a brief screening frailty tool with power calculations for frailty that were not able to be met in terms of patient recruitment. The PROM questionnaires involved detailed questioning of clinical outcomes, whereas the frailty screening tool was a short 4-item questionnaire that was validated and used for screening instead of longitudinal data collection. Despite this, the frailty tool had been identified as the best tool for surgical outcome comparison between SAVR in TAVR in a recent study. This prospective cohort study had reduced number of propensity matched patients, likely related to lack of power, resulting in reduced statistically relevant conclusions.

7.8 A Surgeon's perspective

This 15cm vascular tube that expels and carries blood from the heart is commonly influenced by pathology that affects its structure and function. As a result, this area remains a critical surgical work zone that benefits little from medical therapy or management.

The aortic root and ascending aortic aneurysms are particularly the surgeon's domain and the studies in this thesis have identified that these regions differ both structurally and in relation to risk of rupture from a pressure load. As for the AV, this no longer is the exclusive domain of the surgeon since TAVR has arrived. The studies in this thesis demonstrate that overall clinical outcomes & PROMs between SAVR & TAVR are similar except for certain technique-related issues.

The challenge for surgeons is to improve their techniques in the management of proximal aortic aneurysms & AV disease, and future studies should be directed to this endeavour.

7.9 Future studies

7.9.1 The use of a rupture model to predict Type A aortic dissection

Chapter 2 demonstrated the importance of aortic wall disease/structure in aneurysms. Current recommendations focus only on aortic size without reference to wall structure. Advanced imaging techniques provide some novel information on wall structure and perhaps this can be improved in the future so that collagen/elastin content can be assessed. Perhaps this will involve structural imaging of the vessel wall or functional imaging such as response to wall stress stimuli.

i.e., should structural & functional non-invasive imaging monitoring techniques be used to monitor aortic aneurysms in decision-making for surgery?

Mechanical and computer modelling of the aortic root and ascending aorta has led to improved visualisation of different morphological parameters that were previously not appreciated on plain routine CT imaging. These can be developed to allow better risk stratification of patients and determine the best management for patients with aortic root and ascending aortic pathology.

There is a need to better understand the mechanical consequence of mechanical and geometric variations in the ascending aorta and aortic root to determine better predictive parameters. This is important to identify those patients at higher risk of aortic rupture.

This research would aim to validate a unique 3D aortic model using laboratory based mechanical testing to determine the stress failure of healthy and diseased aorta and aortic roots to identify better predictive parameters of acute and chronic aortic pathology.

This research has the potential to identify areas of susceptibility to aortic root and ascending aortic aneurysms and guide the development of clinical guidelines to monitor progression and to determine the best time to operate on patients with this pathology

7.9.2 The use of 4D flow MRI in predicting clinical outcomes in SAVR and TAVR

Four-dimensional (4D) flow magnetic resonance imaging (MRI) is a novel imaging technique capable of assessing aortic blood flow, and quantifying aortic haemodynamics [217]. However, this remains a surrogate measure and only of value if it is related to clinical outcomes or PROMs. Patients are in search of modalities that are going to improve their symptoms and QOL, and are less interested in what looks better between echo or MRI in SAVR or TAVR. This should therefore remain the pursuit.

Previous studies have reported alterations in aortic wall shear stress distribution and flow eccentricity after both TAVR and SAVR however sample size is small and follow up is limited. Previous studies have demonstrated that changes in the geometry of the AV such as bicuspid valves or AVR result in altered blood flow patterns and parameters. TAVI and AVR with a stented bioprosthesis leads to altered blood flow characteristics in the ascending aorta compared to healthy controls, with more intense flow eccentricity and regional elevation of wall shear stress [218]. Transcatheter AVR results in increased blood flow velocity and WSS in the ascending aorta compared to age- and gender-matched elderly controls. Additionally, TAVR results in altered blood flow eccentricity and displacement in the mid- and distal-ascending aorta, whereas SAVR only results in altered blood flow eccentricity and displacement in the distal ascending aorta (217). Aortic stiffness is increasingly used as an independent predictor of adverse cardiovascular outcomes. Treatment of symptomatic severe AS by SAVR but not TAVR was associated with an increase in aortic stiffness at 6 months [219].

The aims in this research would be to use 4D MRI to determine the stress changes, and turbulent blood flow in the aortic root and ascending aorta following aortic valve replacement. Furthermore,

the aim is to use aortic tissue modelling and 4D MRI testing to determine the stresses applied to the aortic tissues pre and post aortic valve replacement to allow for greater prediction in the management of aortic root and ascending aorta pathology.

This research has the potential to implement new imaging modalities preoperatively and postoperatively in AS and guide earlier management and implementation of treatment for these patients.

7.9.3 The impact of antihypertensives on wall sheer stress in pig models in the management of Type A aortic dissections

Acute aortic dissection is a medical emergency with a range of potentially disabling and fatal complications. The acute management of spontaneous thoracic aortic dissection involves resuscitation and haemodynamic management aimed at minimising further propagation of the dissection by attenuating aortic wall stress and strain.

Aortic wall stress and strain are affected by the rate of change of pressure in the left ventricle and thereby the aorta. The traditional approach to haemodynamic management of these patients is medical stabilisation using beta blockers. Beta-blockers reduce dP/dt, heart rate and blood pressure and society guidelines recommend initial targets of a heart rate less than 60 bpm and a systolic blood pressure between 100 and 120mmHg, which is a Class I recommendation and supported by level C evidence [66].

Beta-blockers may influence aortic wall stress in several ways:

- Direct reduction in dP/dt and velocity of ejection
- Direct reduction in heart rate reduces frequency of aortic distension
- Indirect reduction in dP/dt by reducing heart rate and mitigating the 'staircase' phenomenon
- Increasing shortening velocity by reducing afterload (lower heart rate = lower diastolic blood pressure)
- Increase shortening velocity by increasing end diastolic volume (lower heart rate allows accumulation of greater end diastolic volume)
- Increase distension of the aorta by increasing stroke volume (lower heart rate allows accumulation of greater end diastolic volume)

It is unclear what net effect beta-blockade to a target heart rate of 60bpm has on aortic wall stress and strain, and thereby propagation of an aortic dissection flap.

Existing data identifies that beta-blocker therapy does not reduce systolic wall shear stress or peak velocity in the ascending aorta of patients with BAV [220], however, there is a lack of data regarding the effect of beta-blockers on the haemodynamic behaviour of the ascending aorta.

The role of vasodilator therapy in the care of patients with severe AV dysfunction or aortic pathology has been of considerable interest for some time. Long-term vasodilator therapy with nifedipine was shown to reduce or delay the need for aortic-valve replacement by producing arteriolar vasodilatation, thereby increasing forward flow, and reducing the amount of regurgitation. [221]. Subsequent studies of the aorta itself has drawn considerable interest. In aortic dissection, long-term medical therapy is usually prescribed to decrease the stress on the aortic wall and prevent aortic expansion or rupture [222]. Several animal studies have shown that treatment with an ACEI or ARB slows aortic aneurysm progression and prevents rupture [223, 224]. A randomised clinical trial assessing the use of irbesartan for MFS showed that ARBs decreased aortic expansion [225]. Observational studies show that the use of β-blockers may decrease the aortic dilatation rate in aortic disease [226, 227].

The aim would be to use live pig experiments to determine aortic wall stress and strain measurements at baseline and under conditions of blood pressure control achieved using both beta-blockade and nitrate administration, with comparison undertaken between the two latter conditions.

The results of our studies will provide valuable information on the consequences of beta-blockade on the mechanics of the ascending aorta in a pig model and lay the foundation for human clinical trials to show a delay in aortic enlargement.

7.10 References

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8.1 Appendix

8.1.1 Chapter 2 Publication

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Histological regional analysis of the aortic root and thoracic ascending aorta: a complete analysis of aneurysms from root to arch

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Abstract

Background: Although aortic root and ascending aortic aneurysms are treated the same, they differ in embryological development and pathological processes. This study examines the microscopic structural differences between aortic root and ascending aortic aneurysms, correlating these features to the macroscopic pathophysiological processes.

Methods: We obtained surgical samples from ascending aortic aneurysms (n = 11), aortic root aneurysms (n = 3), and non-aneurysmal patients (n = 7), Aortic collagen and elastin content were examined via histological analysis, and immunohistochemistry techniques used to determine collagen I, III, and IV subtypes. Analysis was via observational features, and colour deconvolution quantification techniques.

Results: Elastin fiber disruption and fragmentation was the most extensive in the proximal aneurysmal regions. Medial fibrosis and collagen density increased in proximal aneurysmal regions and aortic root aneurysms ($p < 0.005$). Collagen I was seen in highest quantity in aortic root aneurysms. Collagen I content was greatest in the sinus tissue regions compared to the valvular and ostial regions ($p < 0.005$) Collagen III and IV quantification did not vary greatly. The most susceptible regions to ultrastructural changes in disease are the proximal ascending aorta and aortic root.

Conclusions: The aortic root differs histologically from the ascending aorta confirming its unique composition in aneurysm pathology. These findings should prompt further evaluation on the influence of this altered structure on function which could potentially guide clinical management.

Keywords: Aortic root, Ascending aorta, Aneurysms, Histology, Immunohistochemistry

Background

Dissection of either the ascending aorta or aortic root results in catastrophic consequences, with an associated high mortality [1]. Aortic aneurysms involving either the ascending aorta or aortic root, predispose patients to aortic dissection [2], but the aortic root aneurysms are especially challenging, given their anatomical location. Consequently, aortic root aneurysms are associated with higher morbidity and mortality compared to those in the ascending aorta [3]. This heterogeneity in outcomes may be attributable to regional structural differences (embryological and histological) within the aortic wall, as well as differences in wall stress. Concerning the latter, ascending aorta pathology is most commonly reported in the right lateral wall where the greatest shear force on the

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aortic wall occurs [4], whereas aortic root pathology is often an extension of the dissection flap into the noncoronary cusp [5]. Despite these structural and functional differences, management of the ascending aorta and aortic aneurysms remains the same [6, 7]. Thus, a greater understanding of aortic wall structure may influence treatments strategies for these heterogenous pathologies.

Normal aortic wall structure

The key microstructural components of the aortic wall are collagen and elastin. With age, the ascending aorta becomes stiffer, with incremental increases in collagen content [8–10]. Similarly, collagen becomes a crucial element within the aortic root, with elastin and collagen fibers in the intermediate layer of the commissures in the annulus [11–15]. The aortic root sinus layers are likened to the ascending aorta itself, with smooth muscle cells, elastic fibers, collagen II and III and proteoglycans within the media, and collagen I makes up the adventitia and intima [16]. The sinotubular junction (STJ) is described as having a thicker wall [17]. The two principal types of collagens found in the aorta are types I and III, accounting for 80–90% of the collagen content [18].

Aortic aneurysm pathology

Historically, pathological analysis of the aortic wall has been primarily observational (i.e. pattern recognition) with limited quantification of the microstructural elements [19]. Reported ascending aorta aneurysm pathology has included cystic medionecrosis, aortitis, varying defects in elasticity, fibrosis, elastin and collagen fiber degradation and transmural defects that seemed to predispose to partial dissections and rupture [20–25]. Aortic root pathology includes cystic medionecrosis, medial fragmentation, elastic fiber and collagen fragmentation, and mucoid accumulation [26, 27]. Direct comparison between regions has described the ascending aorta as having tighter, denser weaves of elastin, and more irregular thickness than in the aortic sinus tissue. Collagen has more of a regular distribution in the ascending aorta compared with the aortic sinuses, and is in greater in proportions on the luminal side in both groups [28]. Observational analysis has shown many similarities between the ascending aorta and root in disease, but notable differences in collagen and elastin structure. Research to date has confirmed that observational analysis has lacked precision and specificity to the core proteins affected. Specifically, histological, and cytological staining by conventional methods loses considerable information, and analysis via biochemical assays and flow cytometry is destructive and morphology is often lost [29]. In addition, digital image analysis, and colour deconvolution is described as being faster, more objective, and less laborious than visual inspection [29]. Digital image analysis has also been supported in determining collagen subtypes in immunohistochemistry [30]. This technique allowed differentiation between collagen types, the assessment of collagen orientation, and was deemed an easily reproducible technique [30]. Regional analysis of histopathology of the ascending aorta and aortic root has not been performed in detail, and no direct comparison have been made [31–34], but there have been reports that collagen types in the aortic root aneurysms change significantly; with collagen I and III decreasing and collagens XI and V increasing [26].

Considering the previously observed structural differences, this project aims to quantify the differences between aortic root and ascending aorta aneurysms in relation to (1) collagen and elastin composition, and (2) collagen subtypes.

Methods

Ethics and governance approval was obtained from the Central Adelaide Local Health Care Network (CALHN) (HREC/18/CALHN/188), with research conducted at the Medical Device Research Institute, and University of Adelaide Histology department, Adelaide, South Australia. Data was collected from July 2019 to September 2020.

A total of 11 human aneurysmal samples were collected over this period (Additional file 1: Table S1), 7 non-aneurysmal samples and 3 isolated aneurysmal aortic root specimens (Additional file 1: Table S2) giving a total of 21 patients. Inclusion criterion was an isolated aortic surgical procedure as a non-emergency. Exclusion criteria included those undergoing a concomitant cardiac or thoracic procedure, or an emergency.

Specimen preparation

Aneurysmal aortic tissue was obtained from the Cardiothoracic Surgical Unit at the Royal Adelaide Hospital, Adelaide, South Australia and non-aneurysmal aortic root and ascending aorta samples were cadaveric hearts obtained from Science Care (Phoenix, Arizona, USA) as part of a tissue donation program. Specimen preparation occurred at the Medical Device Research Institute, Flinders University, and the University of Adelaide Medical School Histology Department. Aneurysmal ascending aortas were sectioned into proximal, middle, and distal regions. Aneurysmal root tissue was excised and separated into sinus and non-sinus (valvular/ostial) regions. Non-aneurysmal regions were cut into root, proximal ascending, mid ascending and distal ascending aorta segments.

Histological and immunohistological preparation

Tissue was placed in 10% neutral buffered formalin solution for fixation following preparation, embedded, and cut using a Leica rotary microtome (Leica Biosystems, Mt Waverley Australia) into 5 μm edge-to-edge sections. The basic histological stains and special stains used included Hematoxylin and Eosin (H&E), Van Gieson (EVG), Massons Trichrome (Massons), Alcian blue, and Von Kossa (VK) stains. Massons' trichrome staining was completed with Celestin blue reagent, stained with biebrich scarlet-acid fuchsin and aniline blue solution, and differentiated in 1% acetic acid. Van Gieson (EVG) staining was oxidised with 0.5% potassium permanganate reagent, decolourised with oxalic acid, stained with miller's elastic stain, and counterstained with Curtis' stain.

For the immunohistochemical component, rabbit polyclonal antibodies to Collagen I (Abcam, Cambridge, UK. Cat # ab138492), Collagen III (Abcam, Cambridge, UK. Cat # ab7778) and Collagen IV (Abcam, Cambridge, UK. Cat # ab6586) were used. In brief, sections were dewaxed using xylene and then dehydrated through alcohols. Dehydrated sections were treated with Methanol/H2O2 for 30 min. The sections were then twice in phosphate buffered saline (PBS) (pH 7.4) for a further 5 min each wash. Antigen retrieval was then performed using Citrate Buffer (pH 6.0), and slides were allowed to cool before being washed twice in PBS (pH 7.4). All slides were then treated with Proteinase K (Merck Millipore, Cambridge, USA. Cat # 21627) for 15 min, then washed with PBS (pH 7.4). Following this process, all slides had non-specific proteins blocked using normal horse serum for 30 min. Collagen I antibody was applied at a dilution of 1/5000, Collagen III at 1/1000 and Collagen IV at 1/500. All antibodies were incubated overnight. The following day, all sections underwent two washes in PBS, then a biotinylated anti-rabbit secondary (Catalogue No. BA-1000, Vector Laboratories, USA) was applied to all sections. They were all incubated for 30 min at room temperature. Following the secondary incubation two PBS washes were carried out, all slides were incubated for a further 1 h at room temperature with a streptavidin-peroxidase conjugate tertiary antibody (Cat No.127, Pierce, USA). Sections were washed under running tap water for 10 min. Sections were visualised using diaminobenzidinetetrahydrochloride (DAB), washed, counterstained with haematoxylin, dehydrated, cleared, and mounted on glass coverslips.

Qualitative analysis

Histological qualitative evaluation was undertaken by the primary investigator and a clinical histopathologist, with the following features particularly noted:

- intimomedial tear (dissecting aneurysm),
- insudation of plasma proteins/erythrocytes (PAS positive),
- elastic fiber disruption/fragmentation/diminution
- medial fibrosis,
- endothelium disruption/loss of integrity,
- thrombosis,
- subendothelial fibrosis,
- mineralization (calcification),
- mural hyalinization,
- mural fibrinoid necrosis,
- mucoid degeneration,
- chondroid metaplasia (cartilage disruption),
- neovascularization,
- cholesterol clefts,
- additional features.

Grading of individual structural components was determined using the classification system recommended by Catell et al., with the degree of pathology denoted as mild, moderate, or severe, and the extension of this pathology denoted as focal, multifocal, or extensive [35].

Quantification analysis

Histological slides were scanned using Nanozoomer digital slide scanner (Hamamatsu Photonics), Zen Blue 3.0 (Zeiss) and NDP view 2.0 (Hamamatsu Photonics) depending on the slide size. Scanned histological slides were then analysed and quantified using Fiji by Image J (National Institutes of Health, USA). Quantification of elastin and collagen fibers then proceeded using the colour deconvolution plugin, whilst collagen type immunohistochemistry proceeded with the immunohistochemistry (IHC toolbox) plugin in Image J v.1.53 (The University of Nottingham, UK). The process involved in the quantification of collagen and elastin fibers included the following steps; image acquisition, scale setting, RGB color space conversion, selection of the colour deconvolution toolbox, adjustment of the threshold value, measurement of the threshold area, quantification of the collagen or elastin fibers in the ROI, and imaging of the collagen and elastin fiber areas. Similarly, the process in quantification of collagen subtypes included; image acquisition, scale setting, RGB colour space conversion, selection of the IHC toolbox, adjustment of the threshold value, measurement of the threshold area, quantification of the collagen subtypes in the ROI, and imaging of the collagen areas. Each measurement was performed twice to minimize quantification errors.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software, San Diego, California). A p-value

of < 0.05 was considered significant. Non-parametric statistical test were utilized considering the skewed population sampled. Specific tests included the Wilcoxon test which was used to compare regional differences between proximal, middle, and distal ascending aorta aneurysms (Additional file 1: Table S9), and the Mann–Whitney U test which was used to compare elastin and collagen content in the aortic root (Additional file 1: Table S13), and collagen subtypes in the aortic root (Additional file 1: Table S20).

Results

Demographics

In the ascending aortic aneurysm group, average age was 65.0 years and there were more males (n = 7) compared to females (n = 4). Reported medical comorbidities were hypertension (9/11), diabetes (3/11) and CVA (1/11). In the aortic root group, average age was 53.5 years and there were two males (n = 2) and one female (n = 1). One valve was bicuspid, and hypertension was the most commonly reported comorbidity (2/3) (Additional file 1: Table S1). In the non-aneurysmal cadaveric group, average age was 73.8 years and there was only one female in the group. Three patients died from cancer related complications, and two from respiratory related complications. Past medical histories were not known beyond the primary and secondary causes of death.

Observational analysis

Intimomedial tearing or extent of the dissection tear was variable amongst each patient depending on the origin of the tear and its extent of its propagation (Additional file 1: Tables S3/S4) (Fig. 1).

Elastic fiber fragmentation, medial fibrosis, thrombosis, and mural hyalinization was greatest in the proximal aneurysmal ascending aorta (Fig. 2). Collagen density was increased in all aneurysmal specimens, confirmed by Von Kossa staining (Fig. 2). Mineralisation and calcification was greatest in the mid ascending aorta in aneurysmal samples. Mucoid degeneration was seen in the proximal aneurysmal regions (Fig. 3) and confirmed by Alcian blue staining.

Chondroid metaplasia, cartilage deposition, protein insudation and cholesterol clefts was also observed to be greater in the proximal segments of the ascending aorta aneurysm specimens, and not present in the aortic root specimens. (Fig. 3).

The most significant additional findings found in the ascending aorta and aortic root aneurysm specimens, were the presence of high-density collagen fibers and lack of elastin fibers on observation. A summary of the basic histology observational findings in the aneurysmal and non-aneurysmal groups is shown in Additional file 1: Tables S3 and S4.

Collagen I was seen in increased density throughout all regions of aneurysmal ascending aorta specimens, with positive blood vessel control, and in the media of the aneurysmal aortic root (Fig. 4). Minimal collagen I was seen in non-aneurysmal samples.

Collagen III stained strongly in the media in most samples and also around the areas of the intimal tearing (Fig. 4). Collagen III was distributed more evenly throughout the aortic root aneurysm samples. Collagen III was scarce in non-aneurysmal samples.

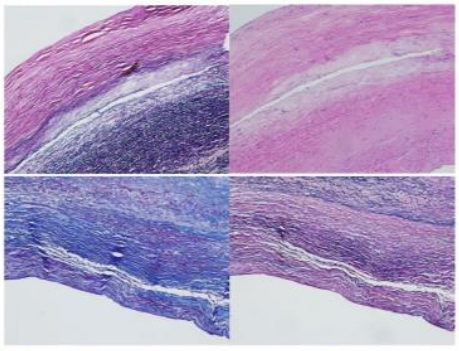

Fig. 1 Ascending aorta aneurysm specimen pictures showing intimomedial tears or dissecting aneurysms (Top left (EVG) and Top right (H&E)). Aneurysms with Massons stain (Bottom left), and EVG aneurysm (Bottom right)

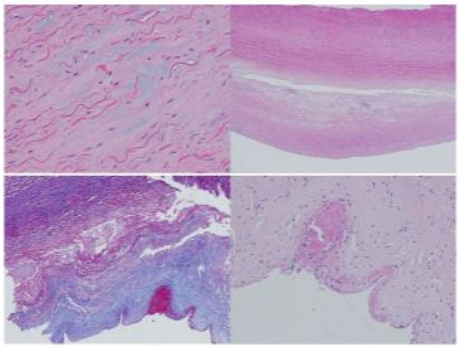

Fig. 2 Elastic fiber disruption and fragmentation in H&E stained segment of proximal ascending aorta aneurysm (Top left). Clear intimomedial tear with complete loss of elastin fiber structure and fibrosis in H&E stained specimen (Top right). EVG (Bottom left) and H&E (Bottom right) stained images showing thrombosis present in the proximal regions of the ascending aortic aneurysm samples

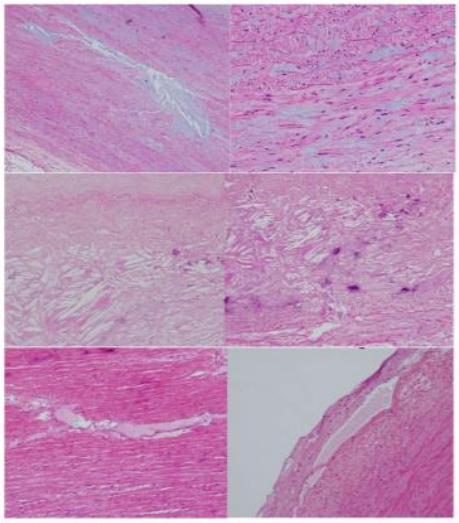

Fig. 3 Mucoid degeneration around proximal ascending aorta aneurysm (Top left) and gross mucoid degeneration in H&E stained aneurysmal sample (Top right). Cholesterol clefts in proximal ascending aorta specimens (Middle left and right). Protein insudation surrounding ascending aorta aneurysms in proximal regions. Seen in H&E images (Bottom left and right)

Collagen IV showed weak generalised staining throughout all ascending aorta aneurysm samples, with increased staining around the intimal tears and positive blood vessel controls (Fig. 5). Increased density of collagen and collagen clumping is seen in all aortic root aneurysm samples (Fig. 5). Collagen IV was scarce in the non-aneurysmal samples. A summary of observational analysis is shown in Additional file 1: Table S5–S8.

Colour deconvolution results

Elastin content showed no clear pattern in aneurysmal versus non-aneurysmal samples. It was higher in aortic root aneurysms (Additional file 1: Table S13) versus non-aneurysms (Additional file 1: Table S11), and regionally highest in the inner parts (Additional file 1: Table S9). Differences were not significantly different (p value = 0.20). (Fig. 6).

Collagen content was clearly higher in proximal ascending aorta aneurysms (Additional file 1: Table S10) versus non-aneurysmal and other regions (p value = 0.0004), as well as higher in aortic root aneurysms (Additional file 1: Table S13) versus non-aneurysmal samples (Additional file 1: Table S12) (p-value = 0.00029).

Immunohistochemistry histological analysis

Collagen I content was low in non-aneurysmal samples (Additional file 1: Table S14), and high in aneurysmal aortic root specimens, particularly in the sinus tissue regions of the root structure (Additional file 1: Table S20) p value = 0.0005). Aneurysmal results are presented in Additional file 1: Table S17 (Fig. 7).

Collagen III content was lowest in the proximal region, and highest in the inner regions in aneurysmal ascending aorta patients (Additional file 1: Table S18) (Fig. 8), but no difference was observed between root regions (Additional file 1: Table S20) p value = 0.44). Non-aneurysmal results are presented in Additional file 1: Table S15.

Collagen IV content did not show any regional variation in ascending aorta aneurysm patients (Additional file 1: Table S19). Content showed great variation amongst root aneurysm samples and between root regions (Additional file 1: Table S20) (Fig. 8) p value = > 0.99. Non-aneurysmal results are presented in Additional file 1: Table S16.

Discussion

This study has shown that collagen content differs between the ascending aorta aneurysms and non-aneurysm samples, with highest collagen content seen in the proximal ascending aorta of aneurysms. Further to this, within the aortic root itself, sinus tissue contains higher collagen content and higher levels of collagen I within it.

Identified imitations included variation in analysis, small number of aortic root patients, reproducible tissue excision from aneurysmal patients, and use of cadavers for normal aortas.

Most previous histological aneurysm studies have focused on BAV aneurysms [28, 36], and dissecting abdominal aneurysms, showing incremental increases in collagen content [32, 37–39], with broken collagen crosslinks and impaired synthesis [40, 41]. Some have reported no change [32, 42]. The increases in collagen deposition and altered collagen synthesis is supported in our findings. Core protein composition in the aneurysmal ascending aorta showed that collagen was extensively distributed, and greater in qualitative and quantitative measurements.

Elastin fiber fragmentation was moderate and extensive in aneurysmal samples, and reduced in quantity as supported by studies suggesting a 50% decrease in diseased samples [32]. Elastic fiber fragmentation and loss [24, 40, 43–45], and decreased elastin content [38, 39, 46] are frequently reported. The ascending aorta has been shown to have tighter and denser elastic properties, is of poorer quality and is thought to be associated with greater compliance under stress [28, 36]. This is supportive of our

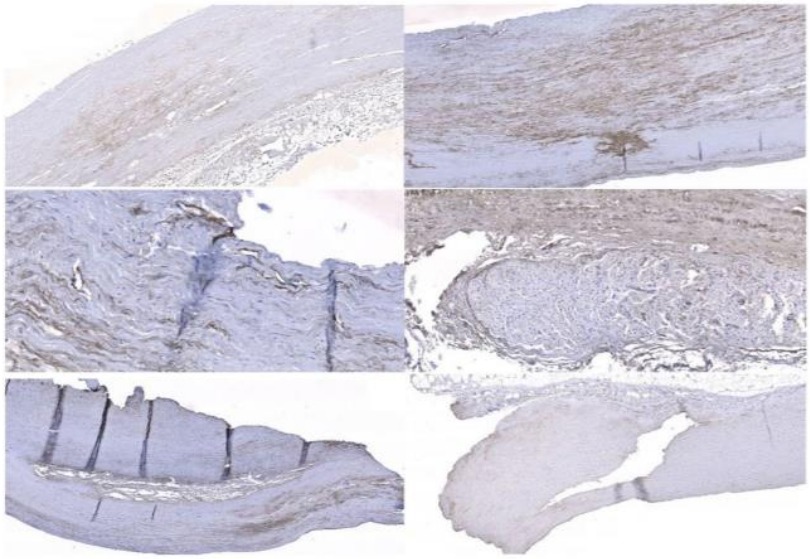

Fig. 4 Increased density of Collagen I (brown staining) in all regions of the aorta, with increased density within the media (Top left and right). Collagen I images in ascending aorta aneurysms showing positive staining around vascular structures (Middle left and right). Collagen III images showing increased antibody uptake around intimal tears and generalised staining within the media (Bottom left and right)

findings of generalized reduced elastin content throughout pathological samples.

The normal aortic root has many complex and variable protein components. Interleaflet triangles contain primarily collagen fibers [26], whereas the sinuses are primarily elastic lamellae [26]. The pathological aortic root results in reduced elastin and fiber fragmentation, as well as decreases in collagen I and III subtypes. An increased collagen amount and decreased concentration is supported in a number of studies looking at dissected aneurysmal aorta's [37–39], contrasted in a study showing a decreased collagen content thought to be related to a weakness in the underlying wall [39].

Detailed studies on the ascending aorta and aortic root aneurysm histopathology (including comparisons) are scarce and therefore comparisons are difficult to make.

Collagen subtypes in the ascending aorta comprise collagen type I, III and IV [26, 39], whereas the aortic root consists of fibrous regions, arterial tissue within the sinuses of Valsalva [47] and is without elastic lamellae [41, 44, 48–50]. Collagen I, III, and IV have been reported in thick bundles and in increased amounts compared to controls [18, 32, 42, 51], with collagen IV shown to be reduced or missing in other aneurysms [18]. The ratio of collagen I and III has been reported as important and reductions in type III collagen have been reported in familial aneurysmal groups [18]. The greatest consistency has been in reporting increases in collagen I and III in media and adventitia of aneurysmal walls [52]. There is great variability in collagen subtypes in aneurysmal and dissection study results with most reporting higher amounts of type I, III and IV in pathology. We report collagen I as having the greatest variability between the root and ascending aorta, but there is no evidence to compare, identifying a significant gap in current knowledge.

Regional analysis found no difference between inner, outer curvature, anterior or posterior regions in the ascending aorta in degree of elastin loss and collagen content [31, 33] but numerous studies reported lateral wall changes [32, 34]. Regional analysis of the root and ascending aorta identified extremes of collagen and elastin in the proximal inner regions, outer regions, and the aortic root itself, suggesting pathological changes occur in these regions more frequently. Comparisons on regional analysis of the aorta are scarce, identifying again a significant gap in current knowledge.

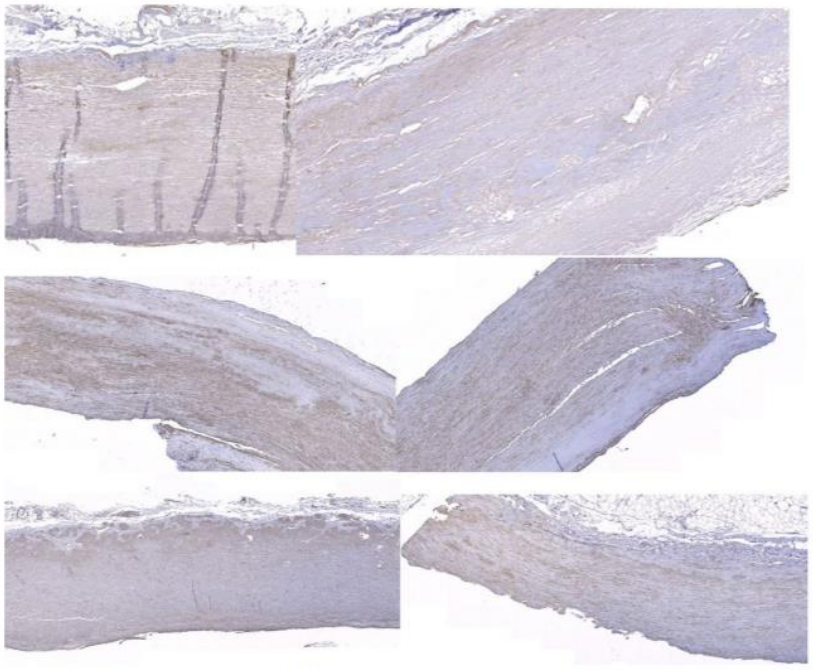

Fig. 5 Generalised Collagen IV staining around ascending aorta aneurysm specimens with positive blood vessel controls (Top left and right). Collagen IV staining showing very generalised staining and increased staining around tears (Middle left and right). Collagen IV staining in the aortic root showing unique clumping of collagen very different to ascending aorta samples (Bottom left and right)

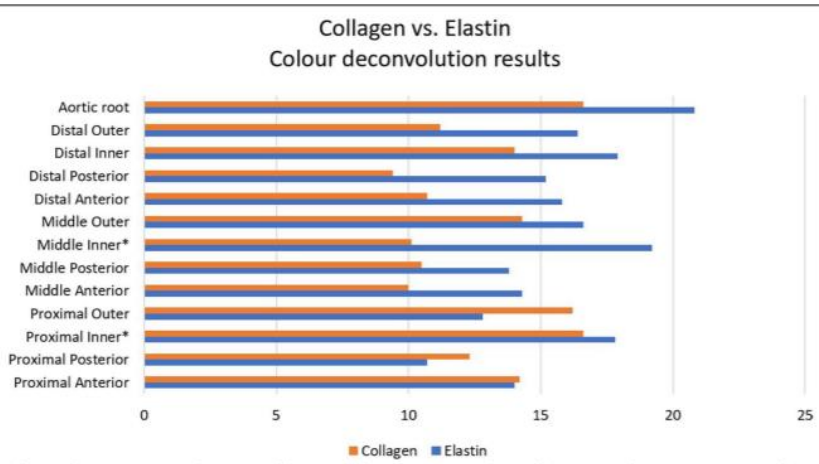

Fig. 6 Colour deconvolution comparison between collagen and elastin in aneurysmal ascending aorta and aortic root regions. * denotes regions of statistical significance

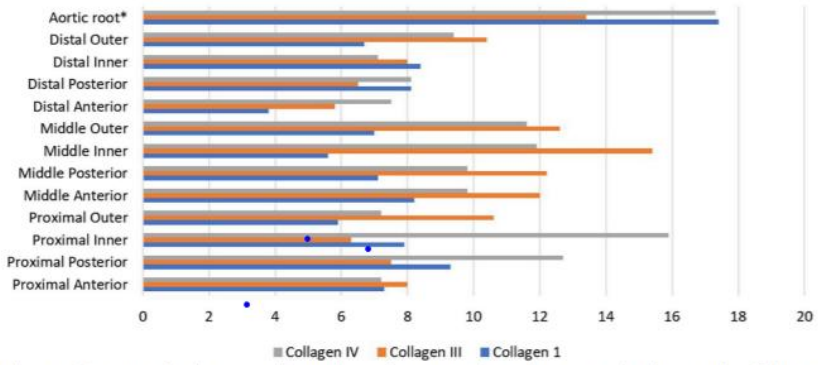

Fig. 7 Colour deconvolution comparison between collagen subtypes in aneurysmal ascending aorta and aortic root regions. * denotes regions of statistical significance

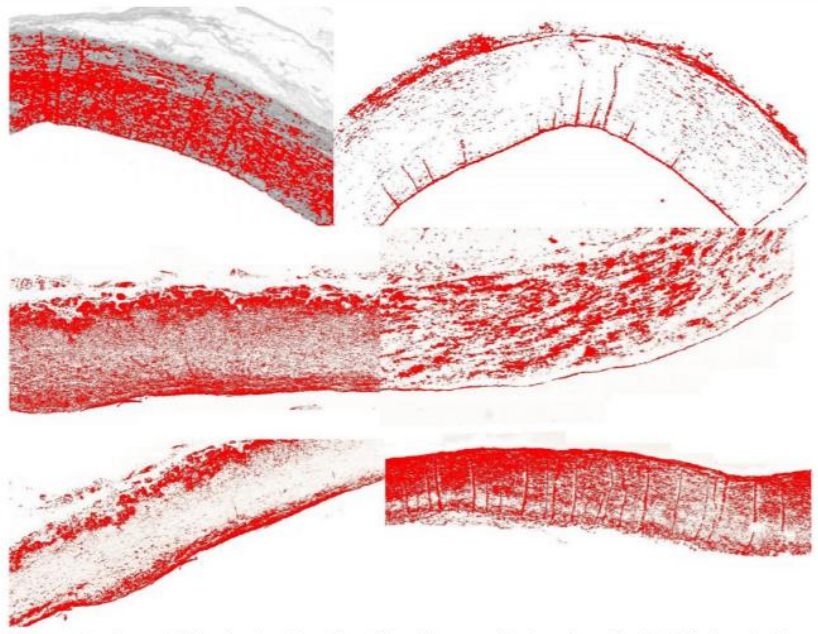

Fig. 8 Colour deconvolution image of EVG stained specimen (Top left) and Massons stained specimen (Top right). Elastin and collagen deposition is marked in red. Aortic root clumping of collagen IV (Middle left and right). Aortic root aneurysm Collagen III distribution (Bottom left) and Collagen I distribution (Bottom right)

Conclusion

We have identified clear microstructural differences between the ascending aorta and aortic root in elastin, collagen, and collagen subtypes. The aneurysmal aortic root appears to show an increased collagen deposition and fibrosis and reduced elastin content in valvular and vascular regions compared to the ascending aorta.

These findings suggest a susceptibility to progressive pathology in the aortic root. Consideration should be given to identification of the root as a unique structure with a response to aneurysmal pathology that differs from all other regions. The authors recognize that increased cases with further isolated aortic root pathology studies with increased sample size are needed to confirm this unique structure and its potential influence on function in disease in future studies.

Abbreviations

STJ: Sinotubular junction; µm: Micro-metres; H&E: Hematoxylin and eosin; EVG: Verhoeff Van Gieson; VK: Von Kossa; H2O2: Hydrogen peroxide; PBS: Phosphate buffered saline; DAB: Diaminobenzidinetetrahydrochloride; PAS: Periodic acid Schiff stain; RGB: Red green blue; ROI: Region of interest; IHC: Immunohistochemistry; CVA: Cerebrovascular accident; BAV: Bicuspid aortic valve.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13019-021-01641-5.

Additional file 1. **Supplementary table 1**. Preoperative demographics, medical comorbidities, and aortic pathology of 11 aortic aneurysm patients. **Supplementary table 2**. Preoperative demographics, medical comorbidities, and aortic pathology of 3 isolated aortic root patients. **Supplementary table 3**. Summary of observational analysis in aneurysmal patients *Boxes filled if not observed. Grade and distribution determined using standardized grading system (53)**. **Supplementary table 4**. Summary of observational analysis in aortic root aneurysm patients *Boxes filled if not observed. **Supplementary table 5**. Summary of observational analysis in non-aneurysmal patients *Boxes filled if not observed. **Supplementary table 6**. Summary of immunohistochemistry observational analysis in aneurysmal patients. **Supplementary table 7**. Summary of immunohistochemistry observational analysis in aortic root aneurysm patients. **Supplementary table 8**. Summary of immunohistochemistry observational analysis in non-aneurysmal patients. **Supplementary table 9**. Summary of colour deconvolution analysis in elastic tissue composition via EVG staining in aneurysmal patients. **Supplementary table 10**. Summary of colour deconvolution analysis in collagen tissue composition via Massons trichrome staining in aneurysmal patients. **Supplementary table 11**. Summary of colour deconvolution analysis in elastic tissue composition via EVG staining in non-aneurysmal patients. **Supplementary table 12**. Summary of colour deconvolution analysis in collagen fiber composition via Massons trichrome staining in non-aneurysmal patients. **Supplementary table 13**. Summary of the colour deconvolution results from the aortic root aneurysm patients. **Supplementary table 14**. Collagen I analysis via colour deconvolution in non-aneurysmal patients. **Supplementary table 15**. Collagen III analysis via colour deconvolution in non-aneurysmal patients. **Supplementary table 16**. Colour IV analysis via colour deconvolution in non-aneurysmal patients. **Supplementary table 17**. Collagen I analysis via colour deconvolution in aneurysmal patients. **Supplementary table 18**. Collagen III analysis via colour deconvolution in aneurysmal patients. **Supplementary table 19**. Collagen IV analysis via colour deconvolution in aneurysmal patients. **Supplementary table 20**. Average immunohistochemistry colour deconvolution results for the isolated aortic root aneurysm specimens.

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Authors' contributions

Equal contribution from TLS, JMA and DO'R in the laboratory testing and manuscript preparation. Equal contribution from JF, JM, JEE, MGW and KR in manuscript review and guidance on final submission. All authors read and approved the final manuscript.

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Availability of data and materials

All data incorporated into manuscript.

Declarations

Ethics approval and consent to participate

Ethics approval and consent to participate was obtained by the patients and relevant ethics and governance institutions. Ethics and governance approval was obtained from the Central Adelaide Local Health Care Network (CALHN) (HREC/18/CALHN/188).

Consent for publication

All co-authors consented for publication. No patient involvement in study requiring consent.

Competing interests

The authors declare that they have no competing interests.

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RESEARCH ARTICLE Open Access

The functional limits of the aneurysmal aortic root. A unique pressure testing apparatus

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Abstract

Background: The aortic root has unique embryological development and is a highly sophisticated and complex structure. In studies that report on the biomechanical characteristics of the thoracic aorta, distinction between the aortic root and ascending aorta regions is nonexistent. Our objective is to determine the maximal pressures at which dissection occurs or tissue failure occurs in the aortic root compared to that of the ascending aorta in the presence of aortic aneurysms. This may help guide preoperative monitoring, diagnosis and the decision for operative intervention for aortic root aneurysms in the normal and susceptible populations.

Methods: We developed a simple aortic root and ascending aorta pressure testing unit in series. Ten fresh porcine hearts were obtained from the local abattoir ($n = 5$ aortic root and $n = 5$ ascending aorta for comparison). Using a saline filled needle and syringe, artificial fluid-filled aneurysms were created between the intima and medial layers of the aortic root. The aorta lumen was then progressively filled with saline solution. Pressure measurement was taken at time of loss of tissue integrity, obvious tissue dissection or aneurysm rupture, and the tissue structure was then visually examined.

Results: In the aortic root, mean maximal pressure (mmHg) at tissue failure was 208 mmHg. Macroscopic examination revealed luminal tears around the coronary ostia in 2/5 specimens, and in all specimens, there was propagation of the dissection in the aortic root in a circumferential direction. In all ascending aorta specimens, the maximal aortic pressures exceeded 300 mmHg without tissue failure or dissection, and eventual apparatus failure.

Conclusion: Our results indicate that the aneurysmal aortic root tissues are at greater risk of rupture and dissection propagation at lower aortic pressure. With further analysis, this could guide clinical and surgical management.

Keywords: Ascending aorta aneurysms, Ascending aortic dissection, Aortic root, Thoracic aorta, Inflation testing

Background

Ascending aortic dissection is the most common catastrophe of the aorta; it is two to three times more common than rupture of the abdominal aorta [1]. Mortality rate of untreated acute dissection involving the ascending aorta is about 1–2% per hour during the first 48 h [2] The first documented case was King George II in 1760 [2]. Constant exposure to high pulsatile pressure and shear stress leads to a weakening of the aortic wall in susceptible patients resulting in an intimal tear [3] Most of these tears take place in the ascending aorta, usually in the right lateral wall where the greatest shear force on the aorta occurs [4].

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Aneurysms of the aortic root arise relatively deep within the heart and because of frequently associated complications, such as aortic insufficiency, present a more complicated problem than the more distal aneurysms of the ascending aorta [5]. The aortic root has unique embryological development and is a highly sophisticated and complex structure. Its optimal structure ensures dynamic behavior in flow characteristics, coronary perfusion and left ventricular function. In studies that report on the biomechanical characteristics of the thoracic aorta, distinction between the aortic root and ascending aorta regions is nonexistent. Aortic root replacement is associated with high mortality and morbidity and is therefore frequently avoided in cases of acute aortic dissection for fear of increased surgical risk. Approximation of the aortic wall layers within the dissected sinuses of Valsalva with a biological glue and subsequent supracoronary aortic replacement offers a simple and efficient method of preserving the native valve and abolishing the aortic insufficiency when it is caused by the distortion of root anatomy. However, non-curative root repair can result in late development of several pathologies, which, especially after use of glue, necessitate challenging redo surgeries [6].

The initial decision regarding the management of the aortic root in type A aortic dissection (TAAD) is whether to repair or replace the dissected sinus segments [7]. The standard indications for aortic root replacement (ARR) in the setting TAAD are extensive tissue destruction, the presence of a concomitant aortic root aneurysm ≥4.5 cm, or a known connective tissue disorder. The most common pathology observed is a primary intimal tear located in the ascending aorta with extension of the dissection flap into the noncoronary cusp, and relative preservation of the left and right coronary sinuses. Rarely are the aortic valve cusps or annulus impacted by the dissection process [7].

A meta-analysis of aortic valve-preserving surgery in acute type A aortic dissection containing 2402 patients from 19 observational studies revealed that, in 95% of the patients, the surgery consisted of conservative root management and supracoronary aortic replacement, while only 5% underwent a curative root repair by valve-sparing root replacement (VSRR) (reimplantation or remodeling). In a large aortic dissection repair centre, 10% of the patients with aortic root dissection, a non-curative root repair using tissue glue was performed at the surgeon's discretion [6].

Coady et al. studied 370 patients with thoracic aneurysms (201 ascending aortic aneurysms), during a mean follow-up of 29.4 months, the incidence of acute dissection or rupture was 8.8% for aneurysms less than 4 cm, 9.5% for aneurysms of 4 to 4.9 cm, 17.8% for 5 to 5.9 cm, and 27.9% for those greater than 6 cm. In this study, the median size of the ascending aortic aneurysm at the time of dissection or rupture was 59 mm. The growth rate ranged from 0.08 cm/yr. for small (4 cm) aneurysms to 0.16 cm/yr. for large (8 cm) aneurysms [8].

The risk of aortic dissection and rupture is often related to the transverse diameter of the aortic sinuses. It is rare with diameters less than 50 mm except in cases of family history of dissection or inpatients with Loyes-Dietz syndrome. Surgery is usually recommended when the diameter of the aortic root reaches 50 mm. Patients with family history of aortic dissection or the diagnosis of Loyes-Dietz syndrome should be operated on when the transverse diameter exceeds 40 mm [8].

Our objective is to determine the maximal pressures at which dissection occurs or tissue failure occurs in the aortic root compared to that of the ascending aorta in the presence of aortic aneurysms. This may help guide preoperative monitoring, diagnosis and the decision for operative intervention for aortic root aneurysms in the normal and susceptible populations.

Methods

We developed a simple aortic root and ascending aorta pressure testing unit in series (Fig. 1). This apparatus consisted of an aortic root and ascending aorta porcine specimen, a pressure transducer measuring in mmHg (National instruments Pty Ltd., Austin, TX), two large vessel clamps, and a 50 ml syringe filled with saline solution with a 21-gauge needle.

Porcine hearts ($n = 5$) were obtained fresh from local abattoirs which included the heart and ascending aorta attached to the brachiocephalic trunk on the right side. In addition, porcine hearts ($n = 5$) were obtained for testing on the ascending aorta alone (excluding the aortic root). Animal ethics approval was not required according to local South Australian Health and Medical Research Institute (SAHMRI) and Preclinical, Imaging, and Research Laboratories (PIRL) protocols.

The aorta was dissected proximally to the left ventricle to include the entire aortic root. The dissection then extended distally to the distal ascending aorta. The proximal limits were the left ventricle and distal limits was the brachiocephalic trunk.

Large vessel clamps were applied to the proximal and distal limits of the aorta (Figs. 2 and 3). The most distal region was limited by the branches of the aortic arch. The most proximal region limited by the left ventricle and careful avoidance of the aortic root structures and left and right coronary arteries. Using a size 11 scalpel blade, a small incision was made in the proximal ascending aorta distal to the aortic root, and the pressure transducer inserted within the ascending aorta lumen. A purse string suture was placed circumferentially around the incision to prevent dislodgement of the transducer

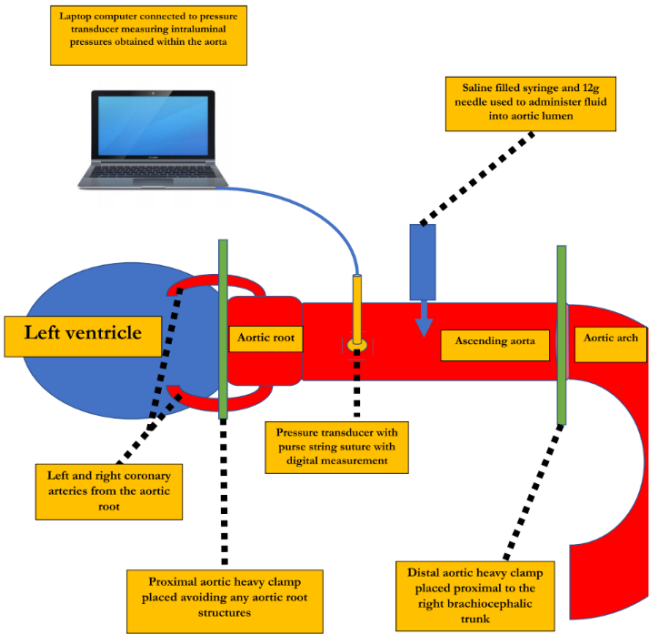

Fig. 1 Diagram of the aortic root and ascending aorta pressure apparatus. This diagram is labelled with the main features of the apparatus. Two clamps are placed proximal and distal to isolate the aortic root and ascending aorta. The administration of saline into the lumen of the aorta and the pressure transducer connected to a nearby laptop to measure and record the maximal pressures before aortic or apparatus failure is demonstrated

during pressurization. The pressure transducer was connected to a laptop computer and pressure measurements taken in real time using LabVIEW (National Instruments Pty Ltd., Austin TX). Saline solution was aspirated into a 50 ml syringe and 21-gauge needle applied. The needle was then inserted between the intimal and medial layers at the level of the coronary ostia to create an aneurysm in the aortic root testing and in the region of the proximal aorta during the ascending aorta testing. Saline solution was administered until a visible aneurysm was created identifying disruption to the tissue layers. Using this same syringe and needle, saline solution was administered into the lumen of the ascending aorta between to distal and proximal clamps until the lumen was filled and pressurized. Concurrent pressure measurements (mmHg) were taken and recorded during filling (Fig. 4). Pressure measurements was taken at time of loss of tissue integrity, obvious tissue dissection or aneurysm rupture. The pressure measurement was determined to be the maximal pressure at time of loss of aortic root tissue integrity. The aortic root and ascending aorta was then opened, and the tissue microstructure was examined visually.

A limitation of this method of creating an aneurysm does not completely mirror the normal, chronic changes of aortic aneurysm formation including the thinning of the tissues, weakening of the connective tissues, and local stress points related to atherosclerosis (penetrating aortic ulcers) which could contribute to the development of aortic dissection.

Results

Pressure measurements were conducted on 5 porcine aortic root specimens, and maximal pressure determined at the time of loss of tissue integrity. The mean maximal pressure (mmHg) at tissue failure was 208 mmHg (see Table 1). Macroscopic examination revealed luminal tears around the coronary ostia in 2/5 specimens (Figs. 5 and 6), and in all specimens, there was propagation of the dissection in the aortic root in a circumferential direction.

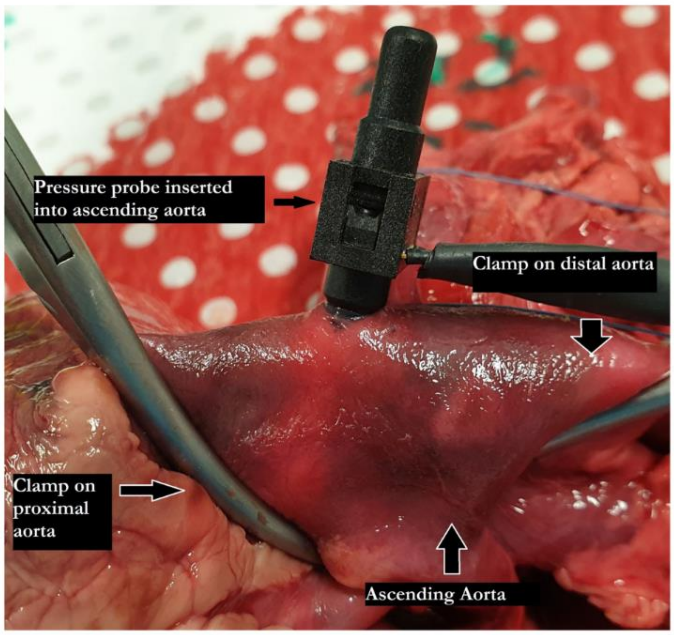

Fig. 2 Aortic root and ascending aorta apparatus photograph. The proximal clamp is sitting at the most proximal portion of the aortic root clear of any aortic root structures. The pressure probe sits at the start of the proximal ascending aorta and distal clamp at the distal ascending aorta. Purse string sutures are yet to be placed around the pressure probe

Pressure measurements were conducted on 5 porcine ascending aorta specimens (excluding the aortic root), and maximal pressures recorded at the time of loss of tissue integrity or apparatus failure (Fig. 7). The median maximal pressure post rupture was 200 mmHg (range 180 to 240), compared to greater than 300 mmHg pre rupture for all specimens. This was significantly different. In all specimens, the maximal aortic pressures exceeded 300 mmHg without tissue failure or dissection, and eventual apparatus failure (see Table 1). Macroscopic examination revealed no luminal tissue dissection or tearing. There was no evidence of aneurysms dissection (Fig. 8).

Discussion

The aortic root is a unique embryological, anatomical and physiological structure that should be distinguished from the ascending aorta in its diagnosis and surgical management of aortic root aneurysms. Diagnosis and subsequent management are determined by aneurysm size, progression of size and predisposing factors such as valvular pathology and genetic conditions such as Marfans syndrome and Loeys-Dietz syndrome. There are no reported studies comparing the macroscopic integrity of the aortic root in times of aneurysm pathology and its propensity to rupture at certain aortic pressures. All studies to date have looked at the aortic root and ascending aorta in section and not as a complete structure reducing its accuracy when compared to physiological conditions [9–15].

It has been reported that many dissection patients do not seem to have markedly dilated aortas at the time of presentation. On review of the International Registry of Aortic Dissections (IRAD) data, of 591 patients reviewed, almost 60% had diameters < 5.5 cm and 40% had aortic diameters < 5 cm. Suggestions have been made for utilization of genetic markers, biomarkers and functional studies to better predict susceptible patients to aortic dissection. If the aortic root represents a unique structure with a predisposition to rupture than the ascending aorta, then do we need even more aggressive monitoring, management and consideration for intervention in aneurysmal proximal ascending aorta and aortic root pathology?

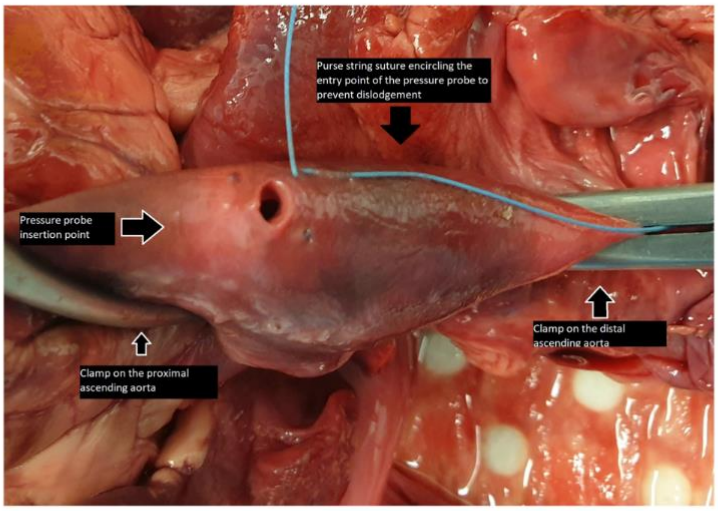

Fig. 3 Overhead view of the aortic root and ascending aorta apparatus. The proximal clamp and distal clamp are at the proximal and distal limits of the thoracic aorta. The purse string suture is placed around the site of the pressure probe in the proximal ascending aorta

We have looked at 10 porcine specimens comparing the aortic root and ascending aorta aneurysm rupture maximal pressures and rupture pattern. There are a number of limitations to our study. First, the use of a clamp at the most proximal part of the aortic root and a clamp in the most distal part of the ascending aorta may cause distortion to the aortic root and affect the pressures recorded. All attempts were made to place the proximal clamp devoid of any aortic root tissue in all experiments. Despite this limitation, clamping allowed for

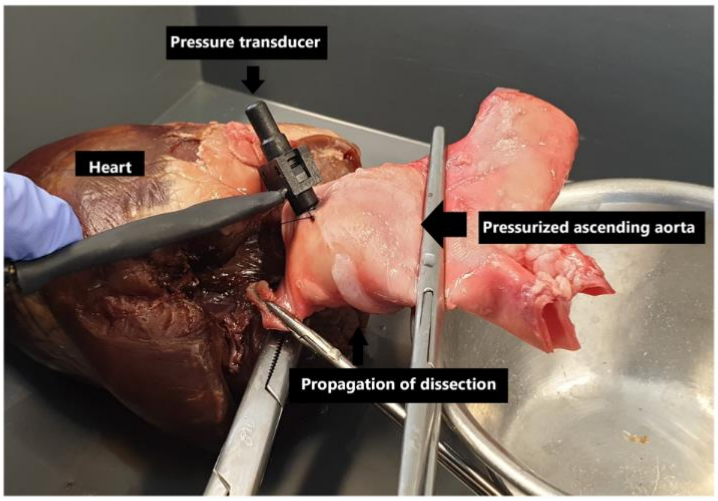

Fig. 4 Photograph showing the aortic root and ascending aorta apparatus during pressure testing. The proximal clamp is positioned proximal to the aortic root, with small clamps placed on the left and right coronary arteries to prevent fluid leak. The pressure probe with associated purse string suture is positioned in the proximal ascending aorta, distal to the coronary arteries

Table 1 Porcine pressure measurements of the aortic root and ascending aorta

Porcine specimen aortic root	Maximal pressure (mmHg)	Macroscopic characteristics	Porcine specimen ascending aorta only	Maximal pressure (mmHg)	Macroscopic characteristics
1	180	• Tissue dissection at site of pressure transducer • Circumferential spread of dissection	**1**	300+	• No loss of tissue integrity • Apparatus failure
2	200	• Tissue dissection at site of pressure transducer • Luminal tear at coronary ostia • Circumferential spread of dissection	**2**	300+	• No loss of tissue integrity • Apparatus failure
3	220	• Tissue dissection at site of pressure transducer • Luminal tear at coronary ostia • Circumferential spread of dissection	**3**	300+	• No loss of tissue integrity • Apparatus failure
4	200	• Tissue dissection at site of pressure transducer • Circumferential spread of dissection	**4**	300+	• No loss of tissue integrity • Apparatus failure
5	240	• Tissue dissection at site of pressure transducer • Circumferential spread of dissection	**5**	300+	• No loss of tissue integrity • Apparatus failure

localization of the maximal pressure to a smaller area and precise administration of luminal fluid. Additionally, the ascending aorta pressure monitor required insertion into the proximal ascending aorta lumen itself causing disruption of the associated tissue structure. Although no major disruption of the tissue occurred at this site under high pressures, this may have been an area of weakness and minor fluid leak resulting in some skewing of obtained results.

Second, due to fresh porcine abattoir animal preparation prior to testing, significant mechanical injury was seen in the cardiac muscle and subsequently not amenable to use in the testing process. This required placement of the proximal clamp to prevent leaking of the intraluminal fluid through the cardiac internal and external tears.

Third, this static pressure model may not reflect the beating heart velocity of ventricular contraction (dp/dT) changes that occur in a clinical setting, but more reflects a measure of the pressure differences and tissue changes that occur under high luminal pressures in different parts of the thoracic aorta.

Simulation models have focused on a number of areas around the thoracic aorta including valvular function, aortic aneurysms, and aortic dissections [10, 16–24]. The studies listed have reported on the flow characteristics around the aortic valve particularly in patients with bicuspid valves and its effects on hemodynamics. Other

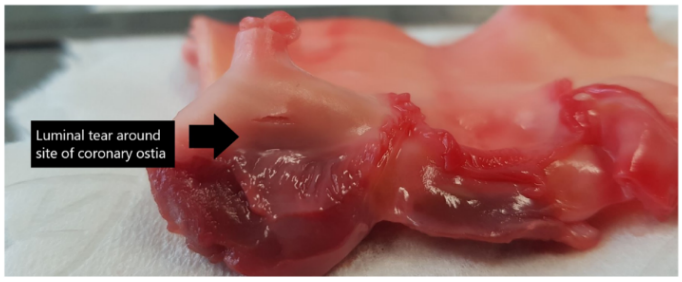

Fig. 5 Photographs of the aortic root region cut open to examine the internal structures. What both photographs show are small tears in the lumen in the coronary ostia and sinus tissue regions as shown by the black arrow. The remaining valvular apparatus remained intact

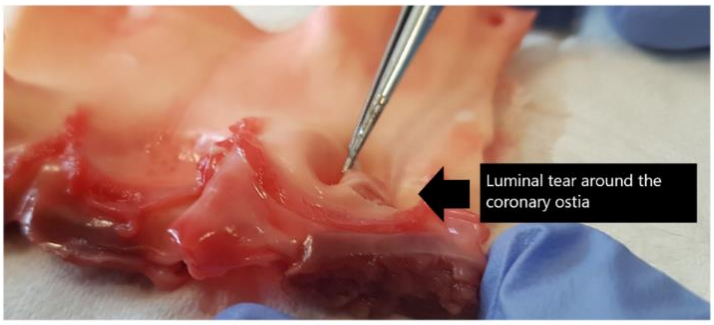

Fig. 6 Photographs of the aortic root region cut open to examine the internal structures. What both photographs show are small tears in the lumen in the coronary ostia and sinus tissue regions as shown by the black arrow. The remaining valvular apparatus remained intact

simulations have focused on reproduction of the aortic aneurysm and dissection process using 3-dimensional (3D) aortic models derived from computer tomography (CT) scanning. Zannoli and colleagues in 2002, 2004, and most recently in 2007 [23] created a mechanical simulator to mock the cardiovascular system reproducing the frank-starling mechanism. Using a balloon and adjustable external reservoir with the aorta simulated by a rubber tubing, they aimed to create a device to reduce the high mortality in the presurgical phase of aortic dissections. They did this by three main mechanisms, improving coronary perfusion, slowing the dissection process, and recovering some of the mechanical efficiency of the cardiac-arterial junction [23]. The disadvantage of such approaches is the associated complexity and resources required to produce these models as well as the lack of gold standard validation in a number of cases.

Our results indicate that the aneurysmal aortic root tissues are at greater risk of rupture and dissection propagation at lower aortic pressure. Future testing of aortic root and ascending aorta pressure limits should include the incorporation of a dynamic pressure model using dp/dt and frank starling forces to replicate the

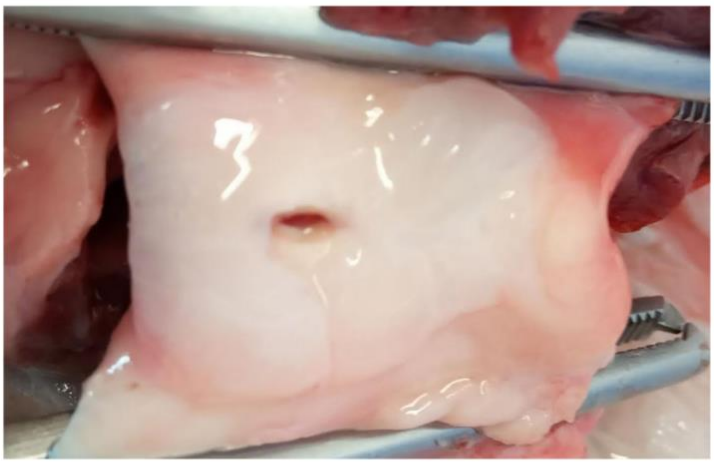

Fig. 7 Photograph of the aortic root region taken from the superior aspect. The spreading of the injected saline into the aortic layers and propagating as a dissection in a circumferential pattern is seen. The superior clamp is proximal and the inferior clamp is distal. The pressure probe is removed from the center of the image for clarity

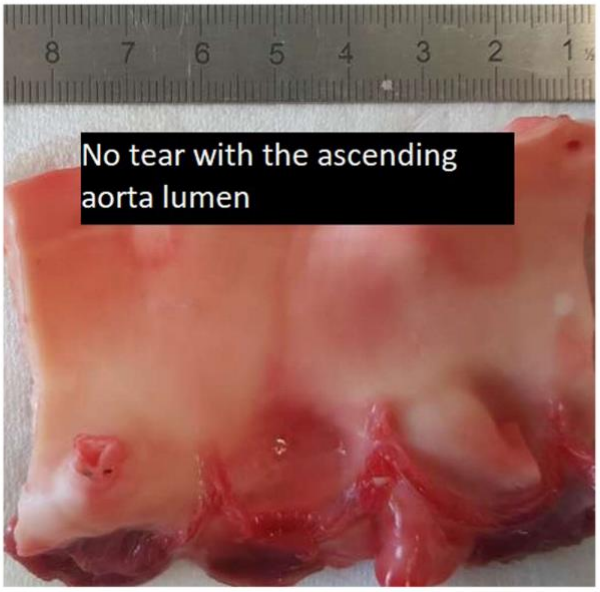

Fig. 8 Photograph of the internal structures of the aortic root and ascending aorta following pressure testing. The photographs show an intact ascending aorta lumen with no tearing of the ascending aortic tissue in this test sample

cardiac cycle as accurately as possible. Further testing of greater tissue numbers is needed to confirm these findings, but consideration should be for much closer monitoring of aortic root aneurysms, strict blood pressure control of patients with known aortic root aneurysms and earlier intervention of aortic root aneurysms.

Conclusion

The aortic root is a unique embryological, anatomical, and physiological structure that is shown to have specific development and progression of aneurysms, and as a result surgical management is different to that of the ascending aorta. No studies to date have tested the limitations of the weakened aortic root tissue, and we have reported on a reliable and reproducible aortic pressure model to identify the differences between these two structures. Knowledge in the pressure and structural limitations of the aneurysmal aortic root could guide clinical management of patients with known aneurysms, monitoring of progression and growth of aneurysms and ultimately surgical repair and replacement.

Abbreviations

TAAD: Type A Aortic Dissection; ARR: Aortic root replacement; VSRR: Valve sparing root replacement; SAHMRI: South Australian Health and Medical Research Institute; PIRL: Preclinical, Imaging, and Research Laboratories; IRAD: International Registry of Aortic Dissections; Dp/dT: Derivative of pressure over time as a measure of cardiac contraction; CT: Computer tomography; 3D: 3-dimensional

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Authors' contributions

Equal contribution from TL Surman, JM Abrahams and D O'Rourke in the laboratory testing and, manuscript preparation. Equal contribution from JE Edwards, MG Worthington and K Reynolds in manuscript review and guidance on final submission. The authors read and approved the final manuscript.

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Consent for publication

All co-authors consented for publication. No patient involvement in study requiring consent.

Competing interests

Nil competing interests from listed authors.

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The susceptibility of the aortic root: porcine aortic rupture testing under cardiopulmonary bypass

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Abstract

Background: In our earlier study on the functional limits of the aneurysmal aortic root we determined the pig root is susceptible to failure at high aortic pressures levels. We established a pig rupture model using cardiopulmonary bypass to determine the most susceptible region of the aortic root under the highest pressures achievable using continuous flow, and what changes occur in these regions on a macroscopic and histological level. This information may help guide clinical management of aortic root and ascending aorta pathology.

Methods: Five pigs underwent 4D flow MRI imaging pre surgery to determine vasopressor induced wall sheer stress and flow parameters. All pigs were then placed on cardiopulmonary bypass (CPB) via median sternotomy, and maximal aortic root and ascending aorta flows were initiated until rupture or failure, to determine the most susceptible region of the aorta. The heart was explanted and analysed histologically to determine if histological changes mirror the macroscopic observations.

Results: The magnetic resonance imaging (MRI) aortic flow and wall sheer stress (WSS) increased significantly in all regions of the aorta, and the median maximal pressures obtained during cardiopulmonary bypass was 497 mmHg and median maximal flows was 3.96 L/m. The area of failure in all experiments was the non-coronary cusp of the aortic valve. Collagen and elastin composition (%) was greatest in the proximal regions of the aorta. Collagen I and III showed greatest content in the inner aortic root and ascending aorta regions.

Conclusions: This unique porcine model shows that the aortic root is most susceptible to failure at high continuous aortic pressures, supported histologically by different changes in collagen content and subtypes in the aortic root. With further analysis, this information could guide management of the aortic root in disease.

Keywords: Aortic aneurysms, Cardiopulmonary bypass, Animal model, Histology, Wall sheer stress

Background

In the realm of aortic root and ascending aorta aneurysm management, it remains unclear of their independent propensity to dissect or rupture under differing influencing factors. A number of animal models have been produced that have aimed to reproduce normal physiology (Table 1) however from our knowledge no animal model has replicated high aortic pressures beyond

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Table 1 Porcine models utilizing cardiopulmonary bypass

Author	Purpose	Methodology	Findings
Angelos et al. [4]	To determine organ blood flow changes in a swine model using CPB to achieve return of spontaneous circulation (ROSC)	Swine model of 10 pigs placed on CBP following VF cardiac arrest	Low flow cardiopulmonary bypass model produces reproducible high resuscitation rates and ROSC
Bufalari et al. [5]	To determine the most effective practice of left pneumonectomy	Swine model of 11 pigs undergoing left pneumonectomy	The most straightforward procedure required careful dissection of the pulmonary ligament, pulmonary veins, pulmonary artery, and finally bronchus
Eckhouse et al. [6]	To establish a reproducible model of aortic dilatation reproducing what happens in Thoracic abdominal aneurysm's (TAA) development	Descending TAA's were induced in 7 pigs using collagenase and crystalline and tissue analysed	Tissue demonstrates aortic dilatation, aortic medial degeneration, and alterations in MMP/TIMP abundance consistent with TAA formation
Kofidis et al. [7]	To determine the feasibility of transapical cardioscopic surgery in a pig model	Transapical access to the ventricle was obtained in 5 pigs with right mini thoracotomy for central cannulation and CPB	Transapical approach allowed for good exposure and adequate surgical field for mitral valve, and aortic valve access, and atrial ablation and intra-aortic procedures
Lundemeon et al. [8]	To determine the effects of pulsed and non-pulsed CPB on microvascular fluid exchange	A total of 16 pigs were randomized to pulsatile (n = 8) or non-pulsatile (n = 8) CPB	No significant differences in the fluid extravasation rates were present between pulsed and non-pulsed cardiopulmonary bypass perfusion
Mariscal et al. [9]	To describe a surgical technique for swine lung transplantation and postoperative management 3 days postoperatively	Involved development of a protocol based on donor surgery, recipient surgery and postoperative care and sacrifice	This survival model can be used by lung researchers to assess development of primary graft dysfunction (PGD) and to test therapeutic strategies targeting PGD
Mickelson et al. [10]	To develop an alternative to canine models in testing for cardiopulmonary bypass research	15 pigs were divided into three groups to determine the optimum conditions during CPB to avoid complications of fluid shifts, metabolic acidosis, and hemoglobinuria	Determined that optimum blood flow rate for cardiopulmonary bypass in swine is in the range of 175–200 ml/kg min. Hyperosmolar priming solution is beneficial for CPB in swine to reduce fluid shifts, metabolic acidosis, and hemoglobinuria
Nicols et al. [11]	To determine the effect of changing FiO2-concentration on SvO2 in a swine model on CPB	8 mixed-gender swine were placed on CPB with an experimental and control group measuring percentage change in blood flow and oxygen delivery	Results suggest that decreased blood flow adjusting for increased SvO2 associated with high PaO2 did not result in significant reduction in adequacy of perfusion markers for organs studied
Oizumi et al. [1]	Development of a swine model for anatomical thoracoscopic lung segmentectomy training	33 pigs were used over a period of 5 years to train operators on segmentectomy via a hybrid (8) or thoracoscopic (23) approach. 3 pigs were converted to thoracotomy due to hemorrhage	Live swine model was considered a good choice for training surgeons on how to perform a minimally invasive lung segmentectomy in humans
Thalmann et al. [13]	Evaluation of several hybrid approaches for pulmonary valve replacement in a swine model	13 pigs were used using 4 different thoracotomy methods for valve implantation, and 5 cases used median sternotomy	Achieved implantation of 12/13 stented valves of which 41% were in the optimal position and 16% had paravalvular leakage. Lower partial sternotomy provided the best deemed approach

that of which is possible in human subjects to truly test the biomechanical limits of the aortic root and ascending aorta. Repetitive high continuous pressure and shear stress leads to a weakening of the aortic wall in susceptible patients resulting in an intimal tear [2], commonly in the lateral wall [3]. Biomechanical distinction between the aortic root and ascending aorta regions is scarce, yet clinical management of aortic root and ascending aorta pathology remains the same. Our objective is to use a porcine model to replicate the real time stresses placed on the aortic wall and aortic root apparatus under cardiopulmonary bypass and under the influence of vasopressor administration, to show the clinical and radiological effects of the aorta under stress, and determine the areas of greatest susceptibility to failure. We will determine the histological characteristics of acute stresses on the aortic wall between the ascending aorta and aortic root apparatus to determine if the macroscopic and microscopic changes align.

Materials and methods

All investigators complied with the 2011 "Guide for the Care and Use of Laboratory Animals", and approval by the South Australian Health and Medical Research Institute Animal Ethics Committee (SAHMRI AEC).

Animal preparation

Following our pilot study indicating differences between the rupture potential of the aortic root and ascending aorta in porcine aortas [1], 5 female adult pigs were obtained for animal testing. All pigs weighed between 50 and 60 kg and were in good health. All animals had external jugular vein and carotid arterial monitoring placed 2 days prior to the testing. Pigs underwent induction using 3–5 ml intramuscular ketamine, maintenance using 2–3% isoflurane, with ongoing ventilation and flow rate of 3–4 L/min. Ongoing monitoring of mean arterial pressure (MAP), systolic and diastolic blood pressure, heart rate and end title CO2 (etCO2) occurred with all experiments with observations recorded every 15–20 min.

Preoperative MRI imaging

All pigs underwent baseline MRI imaging at normal blood pressure and heart rate hemodynamics. All pigs then received a bolus noradrenaline dose of 5–6 ml at 4 mg/4 mL until systolic blood pressure exceeded 200 mmHg. Each pig then underwent MRI at systolic pressures > 200 mmHg to measure WSS and flow parameters.

All MRI scans were performed using a 3-Tesla Siemens Magnetom Skyra (Siemens Healthcare, Erlangen, Germany) (Fig. 1). The subject was positioned in dorsal recumbency within a custom-made MRI compatible positioning device. The subject's condition during MRI was monitored using invasive blood pressure monitoring and an MRI-safe pulse oximeter. Siemens Works In Progress (WIP) sequence, 4D Phase Contrast Flow (WIP 785A) was employed to quantify time-resolved

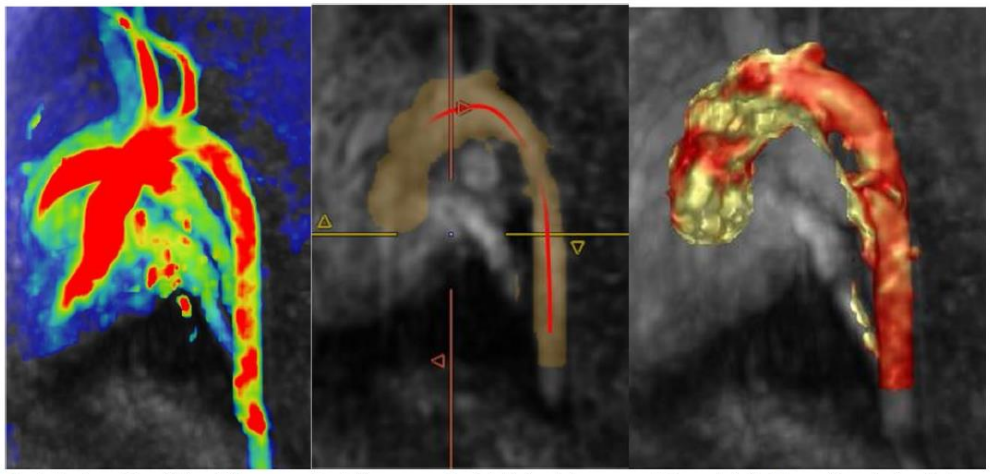

Fig. 1 Porcine subject 4D flow MRI preprocessing (left), segmentation (middle), and the aorta ready for analysis (right) as performed using Circle CVI42 version 5.10.1

flow within the aorta through cartesian sampling in three dimensions. The MRI images were analysed using Circle Cardiovascular Imaging (CVI42) version 5.10.1 Inc, Calgary, Alberta, Canada (Table 2). Each pig study underwent data cropping to identify the area of interest which included the aortic root, ascending aorta, arch and descending aorta. The selected area then underwent preprocessing, whereby a tissue mask is defined. Offset correction and phase anti-aliasing was applied if unwanted flow or noise was identified. The vessel was then segmented, by tracing a centerline from the aortic valve to the descending aorta of which measurement will be determined, and vessel diameter mask adjusted until appropriate for the size of the aorta. Analysis then began with flow measurements. A flow plane is positioned along the center-line until at the appropriate level on the aorta. Each flow plane was positioned at the aortic root, proximal ascending aorta, middle ascending aorta, and distal ascending aorta in which measurements would be taken. Adjustments were made using double oblique views until cross-sectional images were accurately displayed and flow planes aligned. Each measurement was added and flow calculation determined. Net flow (ml/cycle), Peak velocity (cm/s), and regurgitant flow (k) values were calculated automatically. Using the same anatomical plane, wall sheer stress was automatically calculated. Axial maximum WSS (Pa) and Axial average WSS (Pa) was determined.

Animal operation

Following MRI imaging and normalization of pig hemodynamics including heart rate and blood pressure, a cardiopulmonary bypass circuit was created to replicate an adult circuit with a cardiac perfusionist managing its function. Two veterinary assistants monitored and managed the pig throughout the process. Two surgeons were the primary operators for each pig. For all experiments, cardiopulmonary bypass (LivaNova Circuit) was utilized, prepared with a roller pump and inspire oxygenator.

Table 2 MRI phase contrast flow parameters

Siemens Skyra 3T 4D phase contrast flow parameters	
Field of view (FOV)	390 mm × 266 mm
Matrix	176 × 141
Voxel size	2.2 mm × 2.2 mm × 2.2 mm (isotropic)
Repetition time (TR)	40.32 ms
Echo time (TE)	2.29 ms
Velocity encoding (VENC)	180
flip angle	8°
Gating	Retrospective cardiac
Coils	Spine matrix and 18-channel body array

Three-eighths tubing was used to replace the pump header, attached to the autolog reservoir and inspire cardiotomy reservoir and clamped off. Two suckers were utilized for the operative field and primed with 25,000 IU of heparin in 1000 ml of saline. The CPB circuit was primed with 1.6L of saline and 5000 IU of heparin. Operation time for each pig was between 60–120 min. Direct anterior access via a median sternotomy proved to give best access to the aorta and right atrium for cannulation (Figs. 2 and 3). Following heparinization of 15,000 IU, the right atrium was cannulated using a 32f Medtronic venous canula, and the ascending aorta cannulated with a 16f Edwards Lifesciences cannula. Bypass was initiated with good flows, with incremental increases in pressures over the next 10 min. A cross clamp was applied at the distal arch. Cardiopulmonary bypass flows were then increased to maximal flows (L/min) and line pressures, and kept at these measures for 60 s with ongoing monitoring until aortic or cardiac failure. Cardiopulmonary bypass was ceased and euthanasia was performed with 20 ml of intravenous phenobarbitone overdose.

Macroscopic and histological analysis

The aorta was carefully dissected from the left ventricle to the start of the aortic arch in all pigs. Careful attention was made to handling the aorta to ensure no tissue damage was inflicted in this process. from the pig and examined by the two operating surgeons. The aortic root, and ascending aorta were then cut into aortic root, proximal, mid, and distal regions.

Tissue was immediately placed in formalin for fixation following preparation, embedded, and cut using a Leica rotary microtome (Leica Biosystems, Mt Waverley Australia) into 5micro-metre edge-to-edge sections. The basic histological stains and special stains used included Hematoxylin and Eosin (H&E), Van Gieson (EVG), and Massons Trichrome (Massons), Alcian blue, and Von Kossa (VK) stains. Specific immunochemistry antibodies staining for Collagen type I, III and IV were obtained from Abcam Australia Pty Ltd (Melbourne, Victoria, Australia). Anti-Collagen I antibody, Anti-Collagen III antibody, and Anti-Collagen IV antibody were sourced.

Observational analysis proceeded with the primary investigator and a clinical histopathologist. Histological analysis occurred with the use of a double headed microscope at the University of Adelaide Histological department, Adelaide, South Australia.

Histological slides were scanned using Nanozoomer digital slide scanner (Hamamatsu Photonics), Zen Blue 3.0 (Zeiss) and NDP view 2.0 (Hamamatsu Photonics) depending on the slide size. Scanned histological slides were then analysed using Fiji by Image J (National Institutes of Health, USA).

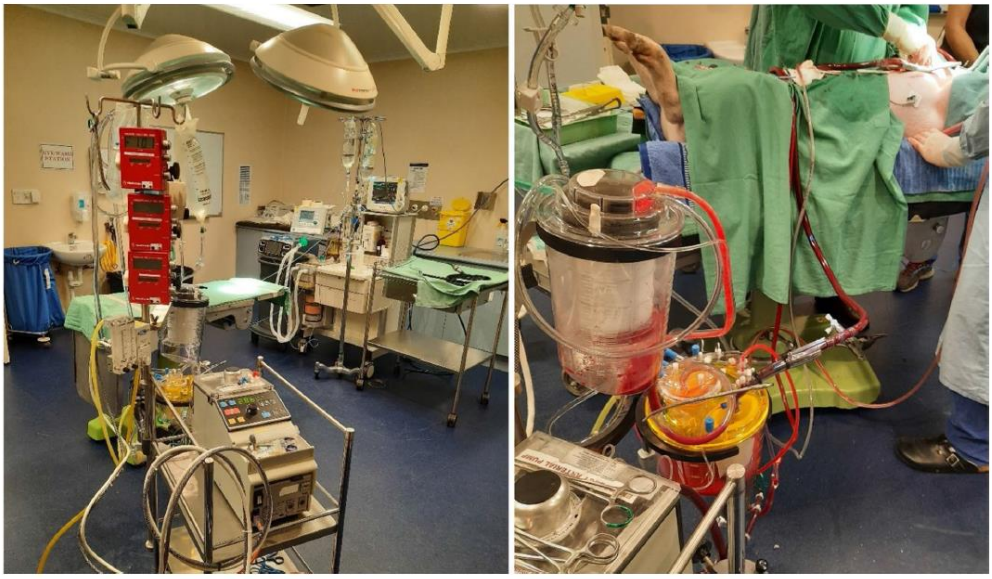

Fig. 2 Cardiopulmonary bypass circuit setup for porcine testing (left), and the active CPB circuit during the porcine experiments (right)

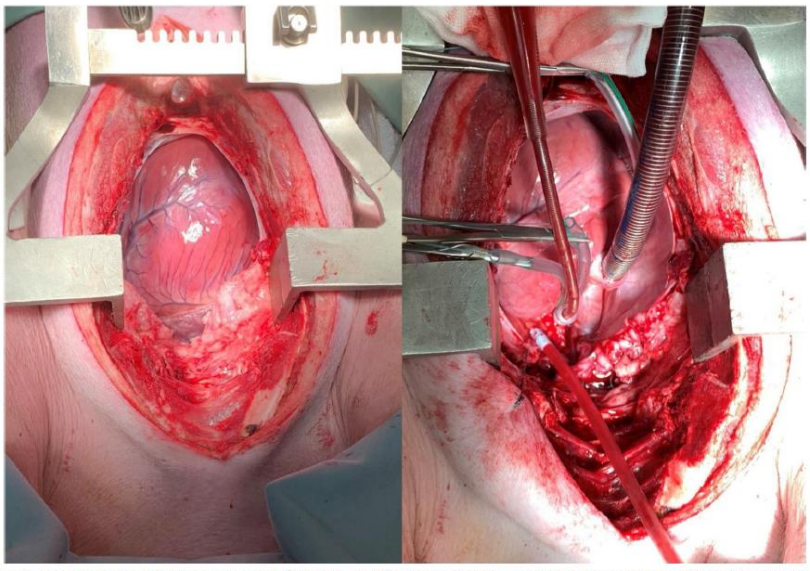

Fig. 3 Median sternotomy and porcine heart exposed (left), and establishment of central cardiopulmonary bypass with porcine subject (right)

Quantification of elastin and collagen fibers then proceeded using the colour deconvolution plugin (IHC toolbox) in Image J v.1.53 (The University of Nottingham, UK). The image was imported into Image J from the NDP or Zen programs, the image cropped to select a region of interest (ROI), and then colour deconvoluted. This ROI then underwent analysis and measurement in Image J to produce a percentage quantification of collagen fibers or elastin fibers within that tissue specimen.

Results

Clinical results

The clinical results from the 5 porcine studies are summarized in Table 3. Median maximal aortic pressures obtained amongst the tested samples was 497 mmHg. The median maximal CPB flows (L/min) was 3.96L. The most common macroscopic findings were aortic cusp hemorrhage and non-coronary cusp tearing which occurred in 4/5 samples (80% of tested cases).

Radiological results

The median max flow (cm/s) in all samples was 79.05 at baseline, and 95.53 following vasopressor. The median wall sheer stress (WSS) (Pa) in all samples was 0.31 at baseline, and 0.48 following vasopressor (Additional file 1: Table 1).

The median max flow (cm/s) at baseline in the aortic root was 53.90, and 64.12 following vasopressor. Median flow in the proximal ascending aorta at baseline was 74.73 and 88.58 following vasopressor. Median flow in the middle ascending aorta at baseline was 84.70 and 101.33 following vasopressor. Median flow in the distal ascending aorta at baseline was 86.37, and 101.95 following vasopressor (Fig. 4) (Additional file 1: Tables 2 and 3).

The median WSS (Pa) at baseline in the aortic root was 0.23, and 0.35 following vasopressor. Median WSS in the proximal ascending aorta at baseline was 0.32 and 0.49 following vasopressor. Median WSS in the mid ascending aorta was 0.37 at baseline, and 0.59 following vasopressor. Median WSS in the distal ascending aorta was 0.31 at baseline, and 0.45 following vasopressor (Additional file 1: Tables 4 and 5).

Although not a direct measure within our study cohort, observational analysis of pathlines pre- and post-administration of vasopressor showed increased vortices flow within the ascending aorta following the administration of vasopressor (Fig. 4).

Histological results

Large tears beneath the non-coronary cusp were noted in all samples (Fig. 5).

The average collagen composition (%) was highest in the proximal inner region (8.48) and proximal outer region (9.08); with other regions having approximately half that of the proximal regions. The average elastin composition (%) was highest in the proximal inner region (23.10). Elastin content was also high in distal and middle inner regions and the aortic root itself compared to other regions (Additional file 1: Tables 6 and 7) (Fig. 6).

General observations were loss of tissue architecture in the aortic root and microhemorrhages in the non-coronary cusp region in all subjects. Immunohistochemistry observations of Collagen I stained specimens showed stronger staining under the intimal layer in all subjects. Collagen III analysis showed diffuse and weak staining

Table 3 Clinical results and macroscopic findings following maximal aortic pressures on CPB

Swine number	Surgical approach	Cannulation	CPB flows (L/min)	Maximal pressure	Macroscopic findings
1	Right thoracotomy	Arterial—ascending aorta Venous—Right atrium	2L	280 mmHg	Valvular failure with no evidence of cusp tearing Cusp hemorrhage present Superior Vena Cava (SVC) tearing resulting in exsanguination of subject
2	Median sternotomy	Arterial—ascending aorta Venous—right atrium	2.2L	286 mmHg	Non-coronary cusp tearing and valvular rupture Cusp hemorrhage Subject euthanized
3	Median sternotomy	Arterial—ascending aorta Venous—right atrium	4.3L	500 mmHg	Non-coronary cusp tearing and valvular rupture Cusp hemorrhage Subject euthanized
4	Median sternotomy	Arterial—ascending aorta Venous—right atrium	5.4L	505 mmHg	Non-coronary cusp tearing and valvular rupture Subject euthanized
5	Median sternotomy	Arterial—ascending aorta Venous—right atrium	3.96L	497 mmHg	Non-coronary cusp tearing and valvular rupture Cusp hemorrhage Subject euthanized
Median			3.96L	497 mmHg	

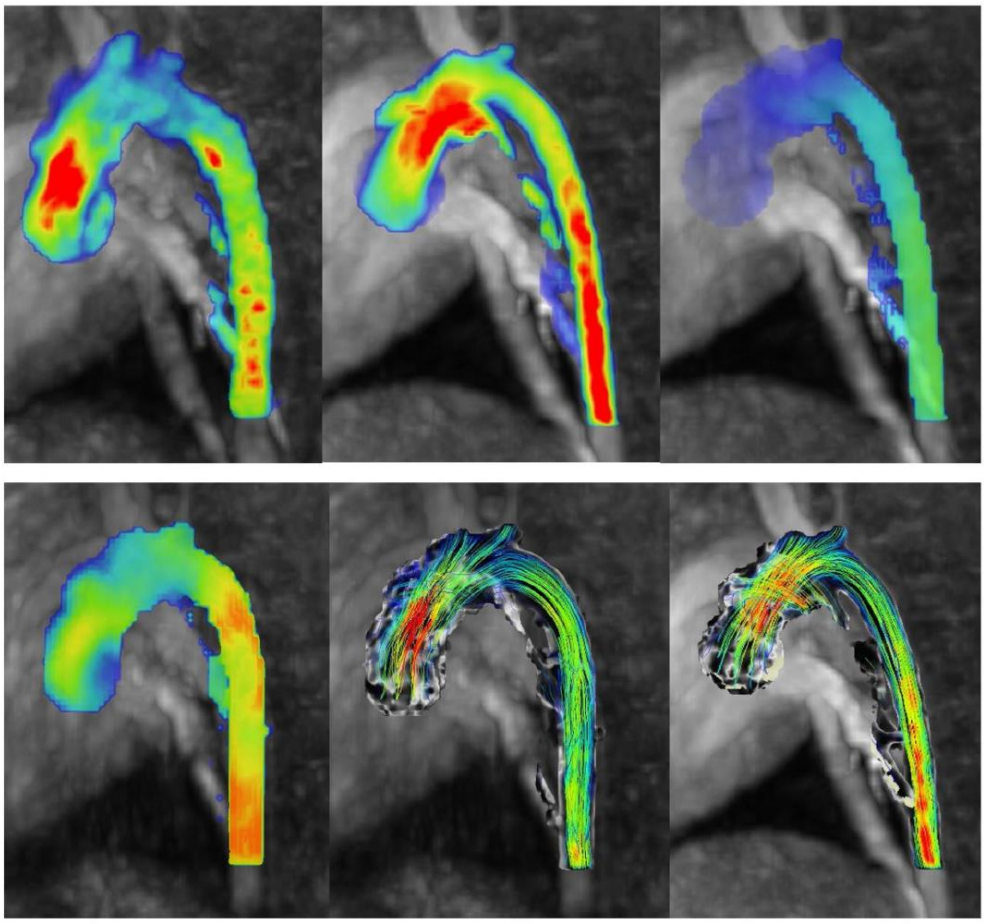

Fig. 4 4D flow MRI imaging results in the porcine subjects. Top left—Porcine flow measurements pre-administration of noradrenaline and Top centre—porcine flow measurements post-administration of noradrenaline. The red shading indicates areas of higher flow measurements (cm/s). Porcine wall sheer stress measurements. Top right—porcine WSS measurements pre-administration of noradrenaline and Bottom left—porcine WSS measurements post-administration of noradrenaline. The areas of yellow-orange-red identify regions of higher WSS (Pa) in ascending order in the porcine subject. Bottom centre—Porcine pathline results pre-administration of noradrenaline and Bottom right—porcine pathline results post-administration of noradrenaline. The pathlines show the direction of blood flow during these stages

in all subjects. Collagen IV analysis showed gross staining with positive blood vessel internal markers within the aortic root in all specimens (Fig. 7).

The average collagen I composition (%) was highest in the distal inner region (28.92), followed by the middle inner (26.15), and proximal outer (25.75) regions. Collagen I was also high in the aortic root (24.53). The average collagen III composition (%) was highest in the middle inner (25.29) and aortic root regions (23.68). The median collagen IV composition (%) was highest in the middle outer (24.51) and proximal anterior (22.35) aorta (Additional file 1: Tables 8–10).

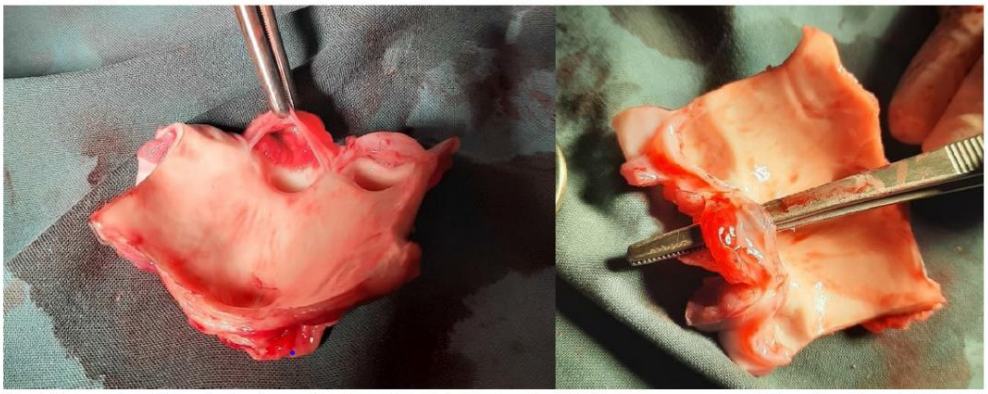

Fig. 5 Photographs of the excised and opened aortic root identifying the tears beneath the non-coronary cusp within each porcine subject tested

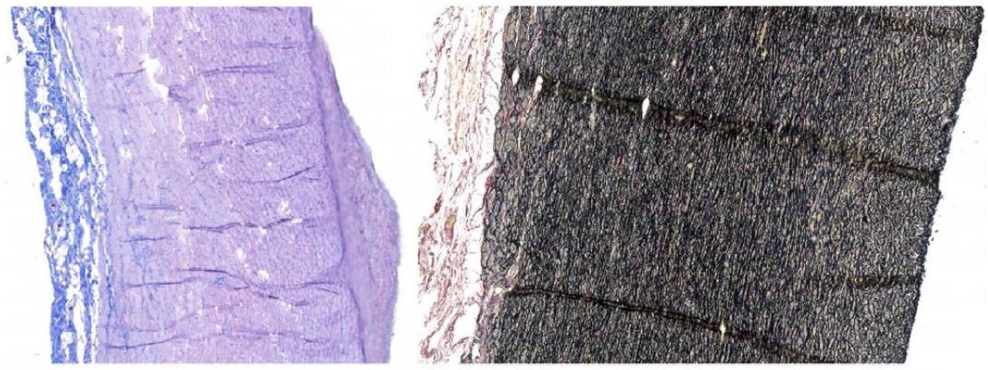

Fig. 6 10 × Massons trichrome staining of the porcine aortic root with darker blue areas indicating collagen deposition (left image). 10 × Van Gieson (EVG) staining of the porcine aortic root with black areas indicating elastin deposition (right image)

Discussion

The aim of this study was to determine what area of the aortic root was most susceptible to failure at high aortic pressures, and how these pressures manifest radiologically and histologically in acute rupture. Using a physiological model, results could identify patterns in radiological, pathological, and histological changes that affect the aorta under stress. Although no studies apart from Surman et al. [1] have reported on the maximal pressures obtained in the porcine aortic root and ascending aorta, many studies have showed that a porcine model is effective in the application of cardiopulmonary bypass and replicating models applicable to human subjects in cardiothoracic surgery [4–13].

Intimal tears are reported to occur mostly in the right lateral wall of the ascending aorta in humans [3], however studies reporting on the most common sites are not well described. Tears affecting the proximal ascending aorta and distal arch have the most catastrophic consequences as they compromise the heart, and brain, respectively. Although our porcine subjects were not aneurysmal, not all dissections and aortic rupture occurs in aneurysmal patients, and therefore the results hold pathological

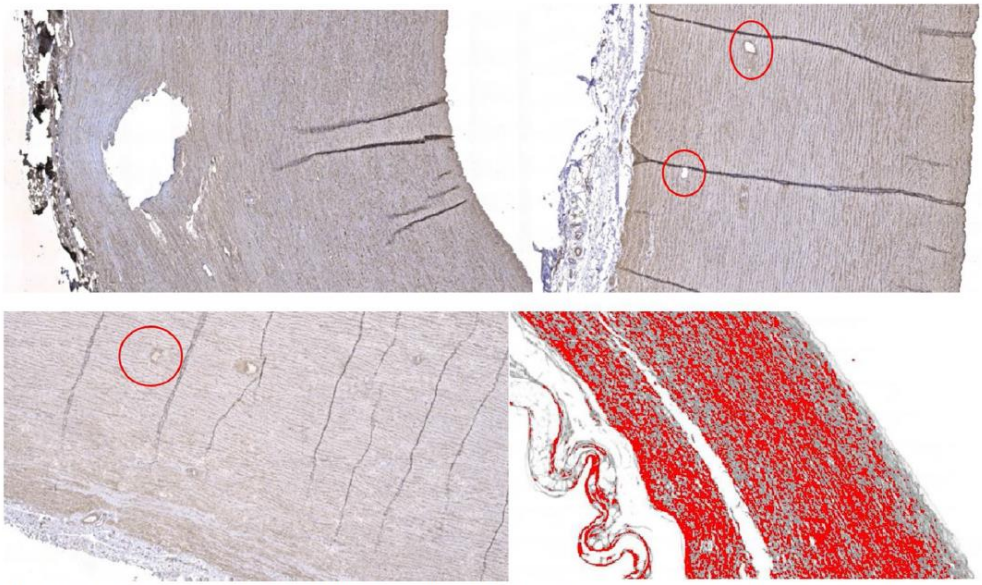

Fig. 7 Immunohistochemistry results showing collagen types within the porcine aortic root and ascending aorta. Top left—Collagen I staining within the porcine proximal aorta as indicated by the brown staining. Top right—Collagen IV antibodies within the porcine ascending aorta noting the positive internal structure staining of blood vessels as highlighted. Bottom left—Collagen IV antibodies within the porcine ascending aorta with positive staining of internal blood vessels as highlighted. Bottom right—Colour deconvolution of immunohistochemistry results showing quantification of Collagen I in the proximal porcine aorta as highlighted by the dense red areas

value in interpretation. When it came to location of the tears, all porcine subjects had splitting beneath the non-coronary cusp and aortic valve failure, identifying it as an area of weakness under high continuous aortic stresses. Surman and colleagues [1] found that the aortic root apparatus in porcine subjects failed at lower pressures compared to the ascending aorta, identifying a clear difference between these two tissues. Clinical findings in this study, supported those findings with failure of the aortic valve apparatus and preservation of the ascending aorta in all regions.

We examined the impact of high intraluminal pressures on the aorta using 4D flow MRI. Median flow measured in cm/s increased significantly, and WSS almost doubled on average across all subjects in all regions of the aorta, identifying that high stresses manifest throughout the aorta from root to distal ascending in only an acute period of time. When we examine the regional changes, the proximal, middle, and distal ascending aorta had significant increase in flow following vasopressor administration indicating that this distribution of increased flow propagates from the root to the arch. Even more profound, was that WSS (Pa) almost doubled in all regions of the aorta following vasopressor administration. The increase in aortic stress was greatest in the mid ascending aorta but high in all regions from the root to the arch. The increase in WSS also correlates to the WSS showing highest increases in the mid and distal ascending aorta groups.

When we review the acute immunohistochemistry and histological changes that result from these acute stresses, we have to determine what is normal before comparing to what is abnormal. The two main types of collagen found in the aorta are types I and III and account for 80–90% of the total collagen, and remaining collagens in lesser amounts [14]. Collagen staining of types I and III was more intense in cases of TAA dissection than in controls and were characterized by thick longitudinal sheets or bundles in the media which were larger than type IV [14, 15], while othersshow collagen proportion in the wall of the dissected and aneurysmal TAA was less than control [16, 17]. Histological and immunohistochemistry analysis in a swine model is not reported in the literature. Interestingly we found that Collagen type I had quite intense staining throughout the intimal layers in all specimens, whereas type III was less abundant. Type IV

collagen is less abundant but in control TAA and normal histological samples of TAA dissection, type IV collagen were seen between the subintimal basement membrane and the media, and in the basement membrane of the adventitia [14].

In our study, collagen IV was prominent in the proximal ascending and aortic root compared to other regions. Eckhouse and colleagues [6] in thoracic abdominal aneurysms in pigs, reported aortic structural changes includingelastic lamellar degradation and decreased collagen content. and colleagues [18] examined differences in aortic sinus tissues between human and pigs. The porcine tissues contain a higher proportion of elastin than the human tissues, which contain a higher proportion of collagen. The elastin fibers in the porcine tissues also appeared to be more undulated than the elastin fibers in the human samples, which were thinner and straighter. This study is limited by the use of a single special stain and lack of quantification of their findings. Collagen I was clearly higher within inner regions across proximal, middle, and distal aortic areas, and similarly collagen III was highest within inner regions including the aortic root. Collagen IV as the least commonly reported type in the thoracic aorta was more equally distributed across regions but showed some higher content in the more middle and proximal regions of the ascending aorta.

Determining protein quantification in porcine tissue is scarce in the literature. A study in 1985 from Davidson and colleagues [19] aimed to determine this in newborn pigs. Relative collagen and elastin syntheses, as a per cent of total protein synthesis, were determined in four separate experiments. Elastin synthesis decreased from about 16.4% in the thoracic aorta to 1.6% of total protein synthesis in the abdominal aorta. Collagen synthesis showed the opposite trend, increasing to 12% of total protein synthesis, although collagen synthesis was still a significant fraction (5–8%) of total protein synthesis in the upper thoracic tissue [19]. Collagen composition was reported as higher in the proximal inner and outer regions of our samples on average across all specimens. Elastin composition was also recorded highest in the inner regions across proximal, middle, and distal aortic regions.

This detailed live animal modelling under conditions of ongoing continuous flow have revealed some important information regarding acute aortic pathology. We have determined that area of greatest risk of failure during high pressure and flow conditions is the non-coronary cusp of the aortic valve within the aortic root apparatus as confirmed by macroscopic and microscopic findings. We have found that the regions of the thoracic ascending aorta under greatest WSS after increased vasopressor insult is the proximal and middle ascending aorta regions.

Histopathology analysis has revealed that the proximal and inner regions of the thoracic ascending aorta have collagen and elastin content that differs from the remaining aortic structure which may predispose or protect it from more chronic insults. When it came to specific collagen content as measured by immunohistochemistry, proximal and inner regions similarly had high collagen I, III, and IV levels but specifically the aortic root had some of the highest collagen I and III levels within the tested samples. We determined that Collagen IV was actually quite a dominant figure in the ascending aorta alone, but was found in minimal amounts in the aortic root.

When we compare the histological and immunohistochemistry analysis of non-aneurysmal samples in pigs and humans there are similarities between quantification values as reported in an upcoming article for publication by Surman and colleagues. When we compare human aneurysmal collagen and elastin quantities, the values are similar between human aneurysmal elastin content and porcine elastin in this study, but the collagen content differs considerably. When we review the human aneurysmal immunohistochemistry versus porcine values in this paper, we see significant differences. The quantity of Collagen I, III and IV are all significantly reduced in human aneurysms compared to non-aneurysmal acute ruptured porcine samples. Reassuringly there is good reproducibility of quantification between porcine and human non-aneurysmal samples as shown in earlier studies [4–13].

Limitations in this study includes histological analysis, whereby immunohistochemistry techniques are limited by the ability of the tissue to take up by the antibodies in question which result in more difficult specimens to analyse and quantify. In addition, pathological analysis and quantification are limited by the investigator and varies considerably with each analysis. This is supported by the results of the same sample shown in the Additional file 1: Tables which shows variation in final percentages following analysis by each investigator of the same sample. Limitations also include the surgical approach. Access and initiation of cardiopulmonary bypass is very challenging and this was shown by difficulties in initial attempts at surgical access. The authors agree that a median sternotomy approach to the ascending aorta is best. Limitations in MRI include long acquisition times, parallel imaging techniques used to compensate (i.e., decreased special and temporal resolution), and the sequence is a WIP so is still investigational.

To our knowledge, this is the first live porcine study measuring the limits and resulting pathology of the aortic root and ascending aorta under high pressures during cardiopulmonary bypass supported by earlier pilot ex-vivo studies [1]. Similarly, no study has quantified

the microscopic details of the aortic root and thoracic ascending aorta following such acute insult.

Conclusion

We have identified that the most vulnerable structure in the aortic root apparatus is the non-coronary cusp of the aortic valve, and the aortic root itself reveals histopathological characteristics such as collagen content and collagen types that differ from the ascending aorta itself, supporting by upcoming histological analysis of human subjects by Surman et al.

These findings further support the idea that the aortic root apparatus, extending up to the inner proximal ascending aorta need to be considered as an independent structure with unique structural susceptibilities and limitations, and protein composition and that should be considered a more vulnerable and delicate structure in surgical management. Further live animal testing in aneurysmal aortas would provide valuable details to these results.

Abbreviations

MRI: Magnetic resonance imaging; 4D: 4-Dimensional; WSS: Wall sheer stress; PIRL: Preclinical, Imaging and Research Laboratories; CPB: Cardiopulmonary bypass; ROSC: Return of spontaneous circulation; TAA: Thoracic abdominal aneurysm; MMP: Matrix metallopeptidases; TIMP: Tissue inhibitor of metalloproteinases; PGD: Primary graft dysfunction; FiO2: Fraction of inspired oxygen; SvO2: Mixed venous oxygen saturation; PaO2: Partial pressure of oxygen; ROI: Region of interest; SVC: Superior Vena Cava; EVG: Van Gieson's stain; WIP: Work in progress.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13019-021-01667-9.

Additional file 1. S1: Summary of radiological results following noradrenaline administration and 4D flow MRI imaging. **S2**: Regional analysis of Flow (cm/s) in 4D flow analysis pre-vasopressor administration. **S3**: Regional analysis of Flow (cm/s) in 4D flow analysis post vasopressor administration. **S4**: Regional analysis of WSS (Pa) in 4D flow analysis pre-vasopressor administration. **S5**: Regional analysis of WSS (Pa) in 4D flow analysis post-vasopressor administration. **S6**: Collagen composition within the sampled tissues via colour deconvolution measurements. **S7**: Elastin composition within the sampled tissues via colour deconvolution measurements. **S8**: Immunohistochemistry results reporting on the percentage of collagen types I in all tissue samples. **S9**: Immunohistochemistry results reporting on the percentage of collagen types III in all tissue samples. **S10**: Immunohistochemistry results reporting on the percentage of collagen types IV in all tissue samples.

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Authors' contributions

The surgical team comprised TLS, JEE, PF, MA, CC, of whom all contributed significantly in the experimental process. Imaging contributions were from GKW, and AW. Histological and Immunohistochemistry analysis contributions were from JF, and JM. The experimental concept and planning was from TLS, JMA and JEE. JBB, and MGW provided equal contribution in review and guidance in the final submission. All authors provided equal contribution in manuscript editing and guidance on the final submission, the the primary author TL Surman completing the submission. All authors read and approved the final manuscript.

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Availability of data and materials

All data incorporated into manuscript.

Declarations

Ethics approval and consent to participate

Animal ethics approval and governance obtained from South Australian Health and Medical Research Institute Animal Ethics Committee (SAHMRI AEC). All investigators complied with the 2011 "Guide for the Care and Use of Laboratory Animals".

Consent for publication

All co-authors consented for publication. No patient involvement in study requiring consent.

Competing interests

The authors declare no competing interests.

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Publisher's Note

8.1.4 Chapter 5 publication

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ORIGINAL ARTICLE

Clinical Outcomes in Surgical and Transcatheter Aortic Valve Replacement: An ANZSCTS Database Review 2001-2019

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Keywords SAVR • TAVR • ANZSCTS • Outcomes

Introduction

The surgical management of aortic stenosis (AS) with an aortic valve replacement (AVR) has been the evidence-based gold standard since 1961 when the first successful AVR was performed. Attempts using the ball-valve prosthesis by Harken and colleagues in 1960 [1] and Starr and Edwards in 1961 [2] resulted in high operative mortality, with 12.2% hospital deaths, and 26.5% total in-hospital and late deaths in 117 aortic valve patients [3]. Since 2002, transcatheter aortic valve replacement (TAVR) has become an evolving option in the management of aortic stenosis, and much of the debate and associated research regarding the management of AS has centred on surgical aortic valve replacement (SAVR) versus TAVR.

Utilising 'real world data' from the Australia & New Zealand Society of Cardiac & Thoracic Surgeons (ANZSCTS) database, the primary objective of this study is to compare the 30-day outcomes (all-case death and permanent stroke) in patients undergoing SAVR and TAVR. Secondary objectives include comparing 30-day outcomes between these groups in relation to deep sternal wound infection and valvular dysfunction, and exploring other early outcomes that greatly impact on patient morbidity and quality of life such as vascular complications and cardiac arrhythmias.

Materials and Methods

Study Population and Design

A comparative cohort study included institutions from 26 public and 32 private participating cardiothoracic surgical units in Australia and New Zealand, including 20 cardiothoracic units that provided the TAVR data (Figure 1).

Ethics and governance approval was obtained from The Central Adelaide Health Care Network (HREC/18/CALHN/188), with approval to utilise the ANZSCTS database remotely via The Safe Haven Environment at Monash University, Melbourne, Australia. From 2001–2019, a total of 15,291 patients were entered into the ANZSCTS database throughout Australia that underwent an AVR. Of these, 14,097 patients underwent SAVR, and 1,194 patients underwent TAVR (Figure 2).

The timeframes for the SAVR and TAVR cohorts were as follows. Data from the SAVR cohort was collected for cases with a date of procedure from the 4 June 2001 to 31 December 2018. Data from the TAVR cohort was collected for cases with a date of procedure from the 16 December 2008 to 20 December 2018. The study period selected was for those available for review from the completed database. There is bias in the data collected. Not all hospitals have

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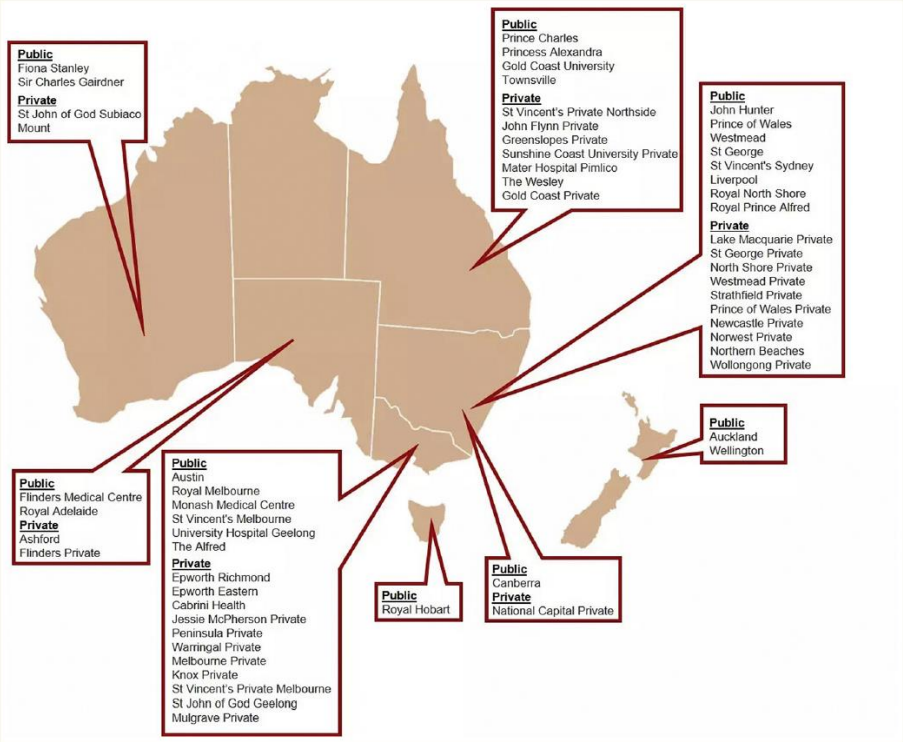

Figure 1 Participating public and private sites in the Australian & New Zealand Society of Cardiac & Thoracic Surgeons (ANZSCTS) database across Australia and New Zealand.

submitted TAVR data, so it is not spread evenly across states. Variation may exist between surgeon-lead TAVR and cardiologist-lead TAVR, and therefore may not be representative of the full range of practice. Missing data was documented and confirmed as a percentage of the total cohort in the following postoperative complications; new renal failure (0.26% SAVR and 0.50% TAVR), permanent stroke (0.32% and 0.57% TAVR), pulmonary embolism (0.32% SAVR and 0.58% TAVR), Deep sternal wound infection (DSWI) (0.45% in SAVR, and 0.42% in TAVR), aortic dissection (3.43% in SAVR and 0.41% in TAVR), acute limb ischaemia (3.43% in SAVR, and 0.41% in TAVR). There is no dropout data as patients are not followed up in this data collection process.

In addition to Australian data, we have reviewed the North American and German experiences with a focus on observational data and trends.

Participant Selection

According to recommended practice by current United States and European guidelines [4], patients underwent SAVR and TAVR procedures after selection by the specific institution's patient recruitment process and heart team discussions. Most surgical patients would have been recruited via standard inpatient or outpatient referral, whereas transcatheter patients were likely to be selected following multidisciplinary heart team review. Patients are typically considered for TAVR if they were deemed to be of a higher operative risk and a transcatheter approach was deemed preferable over open surgical access. Inclusion and exclusion criteria were based on individual institutions guidelines and not protocol driven.

Data was utilised from the ANZSCTS database which records patient demographics, co-morbidities, procedure details, intraoperative data, and postoperative complications up to 30-days post procedure.

The North American experience began in 2010 with the Placement of AoRTic TraNscathetER Valves (PARTNER) I trial for patients who could not undergo aortic valve surgery. A total of 358 patients were enrolled across 21 centres in the United States, with 5-year outcomes being determined on 699 patients across the SAVR and TAVR high risk groups [5].

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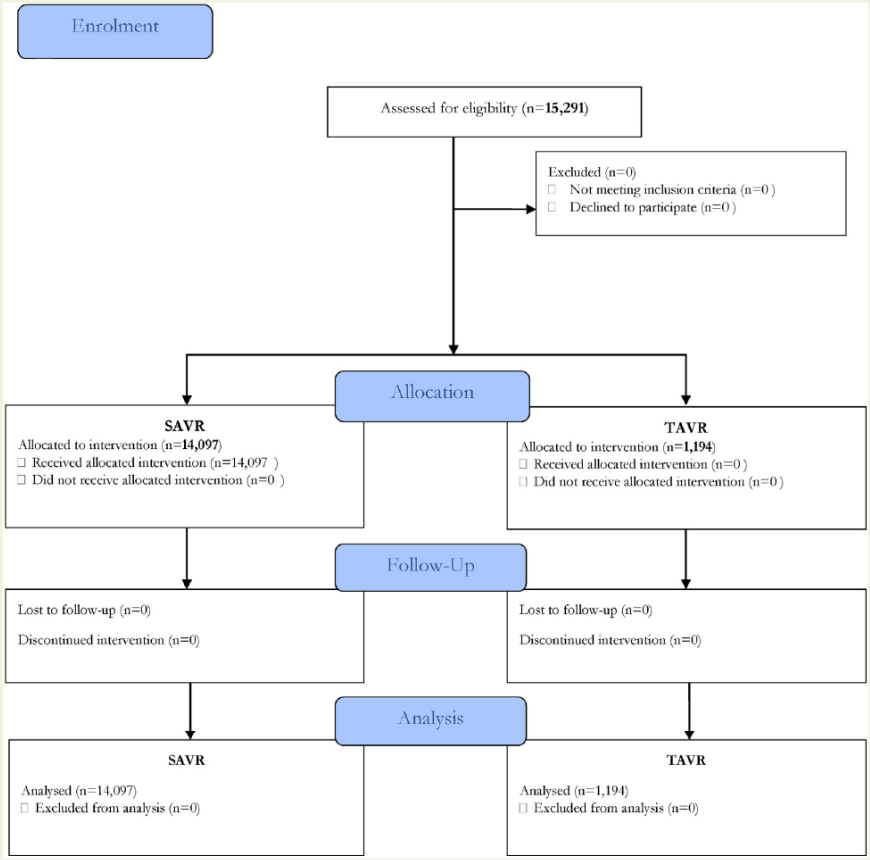

Figure 2 Consort diagram showing recruitment of participants for ANZSCTS cohort study.
Abbreviations: ANZSCTS, Australian & New Zealand Society of Cardiac & Thoracic Surgeons; TAVR, transcatheter aortic valve replacement; SAVR, surgical aortic valve replacement.

Amongst the intermediate risk groups in the PARTNER 2 trial, 2,032 patients were assigned across the two groups for comparison. In the low-risk PARTNER 3 trial, 1,000 patients were randomly assigned to both groups for comparison. The German experience in the aortic valve registry (German Aortic Valve Registry [GARY]) started reporting on outcomes following SAVR and TAVR patients from 1-year results in 13,860 very high-risk patients or inoperable patient [6], 7,613 intermediate risk patients [7], and 20,549 low risk patients [8] in 2019.

Procedure

Of the 14,097 patients who underwent SAVR, a bioprosthetic valve was used in 11,209 cases (79.5%), homo/allograft in 42 cases (0.3%), a mechanical valve was used in 2,675 cases (19%), and 107 cases were unknown (0.8%).

Of the 1,194 patients who underwent TAVR, the procedural access point was greatest via transfemoral access (536, 44.9%), transapical (46, 3.9%), transaortic (23, 1.9%), and trans subclavian (19, 1.6%). The valve types implanted are show below: a summary of TAVR valve types is in Table 1.

All patients in the SAVR and TAVR groups underwent valve replacement only. Cases with concurrent coronary bypass grafting, and other valve procedures were not included.

Study Endpoints

The primary study composite endpoints were 30-day all-cause mortality and permanent stroke persisting for >72 hours peri or postoperatively. Secondary endpoints were:

a) Readmission for deep sternal wound infection within 30 days
b) Readmission for valve dysfunction within 30 days
c) New atrial arrhythmia (atrial fibrillation [AF]or flutter)
d) Heart block requiring implantation of permanent pacemaker (PPM) prior to discharge
e) New ventricular tachycardia of >6 beat run requiring treatment

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Table 1 Transcatheter valve types used in TAVR cohort.

Transcatheter valve type	Total number of valves	Percentage of valves in total cohort
Sapien 3 9600TFX	401	33.6
CoreValve Evolut R	253	21.2
Sapien XT 9300	282	23.6
Portico valve	69	5.8
Sapien 3 S3TF1xx	42	3.5
Corevalve B	37	3.1
Sapien 9000	16	1.3
Evolut Pro	7	0.6
ACURATE neo	5	0.4
SYM-SVxx-002		
Boston Scientific Lotus Valve System – LTV27	4	0.3
Transapical B	1	0.1
Mechanical valve graft prosthesis	1	0.1
Unknown	8	0.7

Abbreviation: TAVR, transcatheter aortic valve replacement.

f) New renal insufficiency (characterised by >200 mmol/0.2 micromol/L increase and a doubling of the preoperative creatinine value or requiring haemofiltration or dialysis)
g) New pulmonary embolism diagnosed by ventilation/perfusion (V/Q scan) or computed tomography (CT) angiogram
h) Continuous coma for >24 hours in a nonsedated patient
i) Pneumonia diagnosed by positive cultures of sputum/aspirate, and haematological or radiological evidence
j) Aortic dissection
k) Anticoagulation complications including bleeding, haemorrhage, and or embolic events related to anticoagulation
l) Septicaemia defined as positive blood cultures and any two of fever, elevated granulocyte, elevated and increasing c-reactive protein (CRP), and elevated and increasing erythrocyte sedimentation rate (ESR) postoperatively
m) Acute limb ischaemia
n) Multi-organ dysfunction involving two or more major organ systems for >48 hours
o) Gastrointestinal tract (GIT) complications postoperatively including GI bleeding, pancreatitis, cholecystitis, ischaemia, hepatitis, or other GI complication
p) And re-intubation

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). A p-value of <0.05 was considered significant. Comparisons between groups was determined using the N-1 chi squared test, which is deemed to have reduced type I errors and increased power compared to others and is recommended where all expected numbers are at least 1, with t-test used for continuous variables. Logistical regression followed by propensity score matching was performed using SPSS statistics 28.01 (SPSS, IBM Corp., Armonk, NY, USA). We performed a stepwise logistical regression analysis using all known patient preoperative demographics and co-morbidities that were collected as part of the ANZSCTS database. The dependent variable was 30-day mortality. Propensity matching was subsequently performed using the outcome of the logistical regression analysis with a tolerance of up to 1.

Results

Preoperative demographics, co-morbidities, and cardiac function of patients from the entire cohort are displayed in Tables 2 and 3. The study endpoints are reported in Table 4.

Compared to SAVR, the TAVR patients were typically older, there were more males than females in both groups, and the mean BMI was similar in both as shown in Table 2. The mean implanted valve size was 27 mm in SAVR and 23 mm in TAVR as shown in Table 2 and valve size details are shown in Table 3. Tissue valve implantation was most common and transfemoral access most common in the TAVR group which probably reflects some bias in the registry.

Patient preoperative risk factors between SAVR and TAVR can be summarised in Table 2. Patients in the TAVR group had more previous CVA's and MI's while a significant portion of SAVR patients had had previous cardiac surgery. The TAVR patients presented with more symptoms, but both SAVR and TAVR equally presented with higher numbers of patients with preserved ejection fractions (EF >60%).

The primary composite end points of 30-day all-cause mortality and permanent stroke showed now significant difference between the groups (Table 4).

The complications between SAVR and TAVR were reviewed, and the major differences identified (Table 5). Heart block requiring PPM insertion was significantly higher in the TAVR group, while new postoperative arrhythmia, particularly AF or flutter was common in SAVR. Re-intubation was higher in the SAVR group, while vascular complications including aortic dissection and acute limb ischaemia were more prevalent in the TAVR group and statistically significant.

Following the application of all variables for this logistical regression for propensity score model there was only 22 subjects within each group that could be matched with a matched tolerance of 1.

Using logistical regression when all patient factors considered for all patients who had SAVR and TAVR, the only preoperative factors that had an impact on 30-day mortality was cerebrovascular disease, respiratory disease,

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Table 2 Preoperative demographics and early postoperative outcomes of entire cohort. Definitions listed in study endpoint section*.

Patient Variables	SAVR n=14,097	TAVR n=1,194	P-value
Mean Age			P<0.001
<60	2,865 (20.3%)	10 (0.8%)	
60-70	3,866 (27.4%)	50 (4.2%)	
71-80	5,133 (36.4%)	275 (23.0%)	
81-90	2,174 (15.4%)	859 (71.9%)	
>91	55 (0.4%)	108 (9.0%)	
Gender and Ethnicity			
Male	8,777 (62.3%)	666 (55.8%)	p<0.001
Female	5,320 (37.7%)	528 (44.2%)	p<0.001
ATSI	190 (1.3%)	7 (0.6%)	p=0.0251
Mean BMI	29.48	27.6	p<0.0001
Mean BSA	1.87	1.77	p<0.0001
Mean valve size	26.66	23.42	p<0.0001
Minimum EoA (BSA x 0.85 cm2/m2) to avoid PPM	1.59	1.5	p<0.0001
iEOA		0.84 (Sapien 3 transcatheter valve), 1.12 (Evolut transcatheter valve)	
NYHA			P<0.001
1	3,260	85	
2	5,467	332	
3	4,427	669	
4	787	103	
Left Ventricular Ejection Fraction (LVEF)			
>60%	8,570	596	p<0.0001
46-60%	3,524	368	p<0.0001
30-45%	1,204	178	p<0.0001
<30%	453	44	p=0.3776
Missing	346	8	
Re-do cardiac surgery	1,972 (13.9%)	0 (0%)	p<0.0001
History of smoking	7,151 (50.7%)	555 (46.5%)	p=0.0049
Missing or unknown	26 (0.2%)	31 (2.6%)	p<0.0001
Current smoker	1,143 (8.1%)	32 (2.7%)	p<0.0001
Diabetes	3,443 (24.4%)	392 (32.8%)	p<0.0001
Hypercholesterolaemia	7,609 (53.9%)	825 (69.1%)	p<0.0001
Preoperative dialysis	229 (1.6%)	32 (2.7%)	p=0.0069
Renal transplant	80 (0.6%)	5 (0.4%)	p=0.5069
Hypertension	9,726 (69%)	1,001 (83.8%)	p<0.0001
Cerebrovascular event	1,453 (10.3%)	227 (19%)	p<0.0001
Remote CVA	657 (4.7%)	93 (7.8%)	p<0.0001
Recent CVA	84 (0.6%)	3 (0.3%)	p=0.1285
Peripheral vascular disease	816 (5.8%)	219 (18.3%)	p<0.0001
Respiratory disease	2,147 (15.2%)	262 (21.9%)	p<0.0001
Infective endocarditis	947 (6.7%)	7 (0.6%)	p<0.0001
Active IE	670 (4.7%)	1 (0.1%)	p<0.0001
Family history CAD	1,696 (12%)	65 (5.4%)	p<0.0001
Previous MI	1,094 (7.7%)	228 (19.1%)	p<0.0001
CCF	4,102 (29.1%)	574 (48.1%)	p<0.0001

Abbreviations: CAD, coronary artery disease; CCF, congestive cardiac failure; MI, myocardial infarction; IE, infective endocarditis; CVA, cerebrovascular accident; ATSI, Aboriginal & Torres Strait Islander; BMI, body mass index; BSA, body surface area; PPM, permanent pacemaker; NYHA, New York Heart Association; EoA, effective orifice area; iEoA, indexed effective orifice area; SAVR, surgical aortic valve replacement; TAVR, transcatheter aortic valve replacement.
*Indexed EoA from Medtronic Inc (Sapien 3 transcatheter valve), 1.12 (Evolut transcatheter valve).

Table 3 Valve sizes in the SAVR and TAVR groups. Missing data was not included*.

Cohort	SAVR n=14,097	Average % of cohort		TAVR n=1,194	Average % of cohort
Aortic valve size mm SAVR			Aortic valve size mm TAVR		
19	759	5.38	19	1	0.08
20	72	0.51	20	13	1.09
21	2,969	21.06	21	-	-
22	94	0.67	22	-	-
23	4,549	32.27	23	260	21.78
24	69	0.49	24	-	-
25	3,562	25.27	25	9	0.75
26	105	0.74	26	441	36.93
27	1,504	10.67	27	40	3.35
28	17	0.12	28	-	-
29	284	2.01	29	328	27.47
30	-	-	30	-	-
31	-	-	31	3	0.25
32	-	-	32	2	0.17
33	-	-	33	-	-
34	-	-	34	74	6.20
Total	13,984			1,171	
Missing	113*	0.80		23*	1.93
Mean valve size	**27 mm**			**23 mm**	

Abbreviations: SAVR, surgical aortic valve replacement; TAVR, transcatheter aortic valve replacement.

preoperative dialysis, angina, and hypertension (Table 6). Excluded variables are shown in Table 7.

Comparison between SAVR and TAVR groups from the database was performed on the entire cohort as propensity score matching was not possible.

Discussion

This study has identified significant differences between SAVR and TAVR clinical outcomes, namely that SAVR has greater prevalence of postoperative arrhythmias and re-intubation, whereas TAVR has increased heart block requiring PPM and vascular complications including aortic dissection and acute limb ischaemia. These differences had not been highlighted or shown to be significant in previous randomised controlled trials (RCTs) comparing the two groups. Following propensity matching and identification of impactful preoperative factors, it is likely these included variables impact on post procedure recovery, particularly for patients who have been on cardiopulmonary bypass and whom have had a prolonged recovery period. This particularly is in reference to cerebrovascular disease, respiratory disease, and preoperative dialysis patients.

The most well-known clinical trials reporting on clinical outcomes in TAVR are the partner trials (Table 8) which report on outcomes in SAVR and TAVR patients in low, medium, and high-risk groups, and include prospectively selected patients that are likely to have better outcomes than the 'real world' data from registries.

Several other studies have reviewed the SAVR and TAVR outcomes in all risk groups (Table 9).

Several institutions have reported on their own registries (state based and national) comparing SAVR and TAVR outcomes in the evolving TAVR field (Table 10).

The most recent reviews of outcomes of SAVR and TAVR groups in Australia and worldwide including the PARTNER trial 5-year outcomes are shown in Table 11.

The PARTNER results may not replicate real world outcomes for several limitations that exist when performing a highly controlled RCT that otherwise do not exist in large scale registries, such as a collection of broad data over an extended period, highly controlled inclusion, and exclusion criteria, and outcomes bias. The ANZSCTS database has greater than 95% data completeness for all reported key performance indicators (KPIs) including in-hospital and, 30-day mortality; reoperation for bleeding; new renal insufficiency; deep sternal wound infection, and permanent stroke. Other performance indicators include new cardiac arrhythmias; duration of intensive care unit stay; duration of ventilation; and red blood and non-red blood cell transfusions. The aim of the database is to maintain a high

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Table 4 Primary and secondary study endpoints.

Study Endpoints	SAVR n=14,097	TAVR n=1,194	P-value
Primary Endpoint			
30-day mortality	258 (1.8%)	20 (1.7%)	p=0.700
Permanent stroke	151 (1%)	15 (1.3%)	p=0.553
30-day mortality and permanent stroke	409 (2.9%)	35 (2.9%)	p=1.000
Secondary Endpoints			
Readmission for deep sternal wound infection	44 (0.3%)	0 (0%)	p=0.053
Readmission for valve dysfunction	10 (0.07%)	0 (0%)	p=0.357

Abbreviations: SAVR, surgical aortic valve replacement; TAVR, transcatheter aortic valve replacement.

standard of care for Australian and New Zealand cardiac surgery patients, and this is achieved through peer review of unit performance on a quarterly basis, and the feedback of performance information to sites.

Albeit the two groups have a very different subset of patients (with SAVR patients being much younger at baseline, and with a high amount of re-do cardiac surgery) preoperative risk factors such as hypertension (HTN), previous cerebrovascular accident (CVA), respiratory disease or previous MI were similar. Preoperative LVEF was also similar between groups, with most patients (>50%) having a normal LV function (>60%) despite a range in NYHA symptoms. The differences between the two groups are highlighted in the statistical analysis (Table 1).

Primary endpoints showed no significant difference between patient groups, supporting recent registry trial results [5,6,9,10,12,13,19,20,22]. Secondary endpoints, including the rate of readmission for valvular dysfunction was low in both groups (0.07% in SAVR and 0% in TAVR) and showed no difference (p=0.3573); suggesting that over this period, the degree of aortic insufficiency is not manifesting clinically. This is also supported in the PARTNER trials [5,9,10].

Additional secondary endpoint of readmission for infection showed no difference between groups (p=0.0532) and was not significant in the trials listed above. The rate of paravalvular leak postoperatively has been higher in TAVR groups throughout the analysis of SAVR and TAVR outcomes [5,9,10,25,26]. This data is not captured in either group in this database analysis unfortunately.

The SAVR results of increased re-intubation prevalence is both supported [27] and reported as showing no difference

Table 5 Postoperative complications recorded in SAVR and TAVR groups.

Complication	SAVR n=14,097	% of cohort	TAVR n=1,194	% of cohort	P-value
New arrhythmia	4,625	32.81	187	15.66	p<0.0001
Heart block	421	2.99	89	7.45	p<0.0001
AF/Flutter	4,000	28.37	61	5.11	p<0.0001
Bradyarrhythmia	185	1.31	33	2.74	p<0.0001
New ventricular tachycardia	234	1.66	10	0.84	p=0.0299
New renal insufficiency	645	4.58	31	2.59	p=0.0013
Permanent stroke	151	1.07	15	1.26	p=0.5428
Pulmonary embolism	20	0.14	0	0	p=0.1958
Coma >24 hours	37	0.26	1	0.08	p=0.2280
New deep sternal wound infection (DSWI)	63	0.45	1	0.08	p=0.0580
Pneumonia	473	3.36	38	3.18	p=0.7398
Aortic dissection	6	0.04	4	0.34	p<0.0001
Septicaemia	135	0.96	2	0.17	p=0.0055
Anticoagulation complication	105	0.75	5	0.42	p=0.1966
Acute limb ischaemia	5	0.03	5	0.42	p<0.0001
Re-intubation	252	1.78	6	0.50	p=0.0010
Multi system organ failure	142	1.01	7	0.59	p=0.1566
GIT complications	182	1.29	17	1.42	p=0.7034

Abbreviations: GIT, gastrointestinal tract; AF, atrial fibrillation; SAVR, surgical aortic valve replacement; TAVR, transcatheter aortic valve replacement.

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Table 6 Preoperative variables influencing 30-day mortality outcomes across SAVR and TAVR groups following propensity score matching.

Preoperative Factor	P-value
Cerebrovascular disease	p=0.007
Respiratory disease	p=0.010
Preoperative dialysis	p=0.016
Angina	p=0.031
Hypertension	p=0.048

Abbreviations: SAVR, surgical aortic valve replacement; TAVR, transcatheter aortic valve replacement.

Table 7 Excluded variables following propensity score matching amongst SAVR and TAVR groups.

Preoperative Factor	P-value
Gender	p=0.173
Aboriginal/Torres Strait Islander	p=0.360
Age	p=0.717
History of smoking	p=0.739
Current smoker	p=0.298
Diabetes	p=0.298
Hypercholesterolaemia	p=0.451
Peripheral vascular disease	p=0.622
Previous MI	p=0.116
Congestive cardiac failure	p=0.341
NYHA Class	p=0.944
PPM in-situ	p=0.273

Abbreviations: MI, myocardial infarction; NHYA, New York Heart Association; PPM, permanent pacemaker; SAVR, surgical aortic valve replacement; TAVR, transcatheter aortic valve replacement.

between groups [28]. Acute limb ischaemia was also significantly higher in the TAVR group, and this would be influenced by the route of access chosen. Given most cases are transfemoral then this risk is understood [29] but incidence also depends on the technique used to access the femoral vessels. Acute dissection was also higher in the TAVR group and statistically significant, which carries an incidence of 0.6–1.9% [30] and is thought to result from stiff wire interaction in the ascending aorta, catheter valve injury to the aortic wall by creating an intimal disruption, valve retraction to expose the balloon in balloon-expandable systems, balloon valvuloplasty injury, or post dilatation balloon interaction with the aorta [31].

New postoperative arrhythmia including AF and flutter was higher in SAVR and statistically significant and occurs in up to 65% of patients undergoing open cardiac surgery, and is a known risk factor for mortality [32]. Replacement of the aortic valve can result in conduction defects due to the anatomical proximity of the AV node to the aortic annulus in both SAVR and TAVR groups [33]. Bradyarrhythmias, ventricular tachycardia (VT) episodes, and complete heart block (CHB) were higher in TAVR; showing statistical significance in bradyarrhythmias and new heart block, and has a similar causative factor being the proximity of the conduction pathway, with LBBB occurring in up to 70% of TAVR cases [33]. The rate of CHB remains high in the TAVR group over this long-term analysis. Albeit CHB has been reported to be as high as 33% [25], the rate in this analysis was 7.45% in TAVR compared to 2.99% in SAVR, and this was statistically significant ($p<0.0001$). It was reported in a meta-analysis in 2014 [34] and supported in 2017 [25], that while PPM post procedure was needed, this PPM implantation had no negative impact on patient's survival despite increasing costs. Patients with CHB are vulnerable to decreased perfusion related to symptomatic bradycardia and decreased cardiac output, syncope related falls and head injuries. Other complications of treatment for CHB include pacemaker lead dislodgement, cardiac perforation, and pacemaker associated heart failure in the long term [35,36].

Comparison of this Australian experience to the large Northern American (PARTNER) and GARY experiences over the last 10 years is important because these international registries capture large patient numbers over a range of clinical risk profiles and subsequently observational data and trends cannot be understated.

Rates of death, stroke, vascular complications, need for pacemaker, and moderate or severe paravalvular regurgitation have declined significantly in the TAVR population over the past decade of PARTNER trials [37]. However, some definitions were ambiguous, of limited clinical utility or required updating/extension. For example, if an unplanned percutaneous or surgical procedure did not lead to an adverse outcome it was not considered a major vascular complication. The issue of subclinical and clinical valve thrombosis has been increasingly recognised, with a reported incidence between 7% and 14%, and given expansion of TAVR into low risk-patients, long-term valve durability becomes an issue. Durability will become clearer as we extend into 10-year durability results. In comparison to the Australian experience, there were declines in vascular complications, declines in paravalvular leaks and overall stable rates of new pacemakers postprocedure.

Similar to the PARTNER trials [5,9,10], issue of valve durability remains open in the GARY registries. Patient cohorts recruited in 2018 and 2019 in younger age groups will focus on this long-term durability with echocardiography in the next 10 years. In comparison to the Australian experience and other RCTs including North America, SAVR cohorts have maintained a very low in hospital mortality (2.1%) compared to TAVR (5.1% in transvascular groups) [38]. Severe vital complications (death on the same day, conversion to sternotomy, low cardiac output that required mechanical supports, annular rupture and aortic dissection) occurred in 5% and technical complications were registered at 4.7% in the initial 15,964 TAVR procedures from 2011 to 2014 [38],

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Table 8 Randomised control trial (RCT) PARTNER trials 1-3 comparing SAVR and TAVR outcomes in high intermediate and low risk groups.

Study	Year	Cohort	Outcomes
Mack et al. (PARTNER 1) [5]	2015	High risk SAVR and TAVR (mean STS score 11.5%)	• The primary outcome of the trial was all cause mortality at 1 yr, with secondary endpoints being stroke, readmission, AKI, vascular complications, and bleeding events. • Periprocedural stroke or TIA was higher in TAVR (5.5%) versus SAVR (2.4%), and transapical TAVR had higher mortality compared to transapical SAVR. • At 5 yrs there was no significant difference in all cause or cardiovascular mortality, stroke, or re-admission between SAVR and TAVR. • Moderate or severe aortic regurgitation caused by paravalvular regurgitation was more common in the TAVR group and was associated with lower survival. Author and investigator reasoning for the differences between paravalvular leak and clinical outcomes relate to valve development, operator expertise and experience, and patient selection for such trials.
Leon et al. (PARTNER 2) [9]	2016	Intermediate risk SAVR and TAVR (mean STS score of 5.8)	• The primary outcome of the trial was death of any cause or disabling stroke at 2 yrs, with secondary endpoints being vascular complications, life-threatening bleeding, AKI, new onset AF, re-admissions, PPM implantation, length of stay and paravalvular aortic regurgitation, in addition to others. • There was no significant difference in the primary endpoints of death and disabling stroke between SAVR and TAVR. • At 30 days, vascular complications were more frequent in TAVR (7.9%) vs SAVR (5%). Life-threatening bleeding was reported to have occurred more frequent in SAVR (43%) vs TAVR (10%), as well as new onset AF in SAVR (26%) vs TAVR (9%). The need for PPM was higher in TAVR (8.5%) than in SAVR (6.9%). • The frequency and severity of paravalvular aortic regurgitation was greater after TAVR (22.5% mild and 3.7% severe) vs SAVR. The severity of paravalvular leak worsened at 2 yrs in the TAVR group, and those who had moderate to severe regurgitation had higher mortality within 2 yrs. This was statistically significant with a p=value of <0.001). When explored in more detail, mild paravalvular leak worsened from 30 days to 2 yrs in the TAVR group in a reduced number of patients with supporting echocardiographic findings. • Moderate or severe paravalvular leak worsened from 30 days to 2 yrs in the TAVR group, while in the SAVR group; mild, moderate, or severe paravalvular leak improved over time. • Trial conclusions was that SAVR and TAVR outcomes in respect to death and disabling stroke are similar in intermediate-risk patients, and it was deemed that the TAVR expandable prosthesis may reduce patient prosthetic mismatch and result in greater long-term outcomes; and paravalvular leak resulted in increased mortality in the moderate to severe TAVR group.
Mack et al. (PARTNER 3) [10]	2019	Low risk SAVR and TAVR (mean STS 1.9%)	• The primary outcome was death, stroke or rehospitalisation at 1 yr; with secondary endpoints being new onset AF at 30 days, length of hospital stay, improvement in heart failure symptoms and functional outcomes as measured by a 6-min walk test. • At 1 year, death from any cause was higher in the SAVR group (2.5%) vs the TAVR group (1%). Stroke was higher in the SAVR group (3.1%) vs the TAVR group (1.2%). Rehospitalisation was higher in the SAVR group (11%) vs TAVR group (7.3%). • Heart failure symptoms (NYHA II, III, IV) were reported at 30 days and at 1 year. At 30 days symptoms were worse in SAVR (33.3% of patients versus 19.7%), but at 1-yr symptoms were worse in TAVR at 17.7% of patients vs 16.7% in the SAVR group. • The percentage of mild paravalvular regurgitation at 1 yr was higher in the TAVR group (29.4%) vs the SAVR group (2.1%). • Trial conclusions was that among patients with severe aortic stenosis who were at low risk for death with surgery, the rate of composite of death, stroke, or rehospitalisation at 1 yr was significantly lower with TAVR than with SAVR [10].

Abbreviations: SAVR, surgical aortic valve replacement; TAVR, transcatheter aortic valve replacement; STS, Society of Thoracic Surgeons; AKI, acute kidney injury; TIA, trans ischaemic attack; PPM, permanent pacemaker; AF, atrial fibrillation; NYHA, New York Heart Association; PARTNER, Placement of AoRTic TraNscathetER Valves.

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Table 9 Prospective studies comparting SAVR and TAVR outcomes.

Study	Year	Cohort	Outcomes
Rosato et al. (Observant study) [11]	2016	Low risk SAVR vs TAVR	• Improved 3-yr survival was better in SAVR (83.4%) vs TAVR (72%), and freedom from major cardiac and cerebrovascular events was greater in SAVR (80.9%) vs TAVR (67.3%).
Tyregod et al. (Notion study) [12]	2015	High risk SAVR vs TAVR	• They found no significant difference between SAVR and TAVR in the areas of composite death rate of any cause, stroke, or MI after 1 yr.
Reardon et al. (Surtavi trial) [13]	2017	Intermediate risk SAVR vs TAVR	• The incidence of the primary endpoint (death or disabling stroke at 2 yrs) was 12.6% in the TAVR group and 14% in the SAVR group; with TAVR deemed a suitable non-inferior alternative to SAVR in this patient group.

Abbreviations: SAVR, surgical aortic valve replacement; TAVR, transcatheter aortic valve replacement; NOTION, Nordic Aortic Valve Intervention; OBSERVANT, Observational Study of Effectiveness of SAVR–TAVI Procedures for Severe Aortic Stenosis Treatment; SURTAVI, Surgical Replacement and Transcatheter Aortic Valve Implantation; MI, myocardial infarction.

Table 10 National registries comparing SAVR and TAVR outcomes.

Study	Year	Cohort	Outcomes
Moat et al., Duncan et al. [14,15]	2011	TAVR outcomes	• Reported a 92.9% 30-day survival in patients undergoing TAVR, 1-yr survival was 78.6% and 2-yr survival 73.7% [13]. • Follow-up study on the same group of patients revealed a 3-yr survival of 61% and 5-year survival of 45%, which was deemed respectable [14].
Eltchaninoff et al. (France registry) [16]	2011	High risk SAVR and TAVR outcomes	• Reported a high predictive operative mortality (18.9%) and mean age of 82 yrs reported a 12.7% 30-day operative mortality and initial stroke rate of 3.7%.
Gilard et al. (France 2 trial) [17]	2012	High risk SAVR and TAVR outcomes	• Reported that 30-day operative mortality reduced to 9.7% and 1-yr mortality was 24% in a similar high risk, elderly cohort. The major stroke rate had decreased to 2.3%.
Grant et al. (United Kingdom registry follow-up) [18]	2016	SAVR and TAVR outcomes	• Observed a 30-day mortality of 2.1% in SAVR and 6.2% in TAVR as well as 5-yr survival rates of 82% and 46% respectively.
Hamm et al., Mohr et al., Walther et al. (German aortic valve registry) [6,19,20]	2017	SAVR and TAVR outcomes	• One-year follow-up demonstrated excellent results in the SAVR group, and TAVR was deemed a good alternative for elderly and high-risk patients [18,6]. • Severe complications in TAVR patients have steadily decreased over time [19].
Thourani et al. (Canadian registry) [21]	2017	High risk TAVR outcomes	• Evidenced relatively high mortality rates associated with TAVR in extreme-risk patients at mid-to long term follow up (24% at 1 yr, 56% at 4 yrs).
Brennan et al. (Transcatheter valve registry/STS database) [22]	2017	Intermediate and high risk SAVR vs TAVR	• In both SAVR and TAVR, there was no significant difference in rates of death (17.9% vs 17.3%), and stroke (3.3% vs 4.2%).
Barbanti et al. (The Italian Observant study) [23]	2019	Low risk SAVR vs TAVR	• At 5 years, the rate of death from any cause was 35.8% in SAVR and 48.3% in TAVR (p=0.002). In addition, TAVR was associated with increased risk of major adverse cardiac and cerebrovascular events (54%) vs SAVR (42.5%).
Virtanen et al. (Finn Valve registry) [24]	2019	Low risk SAVR vs TAVR	• Mortality at 30-days was 3.6% in SAVR and 1.3% in TAVR. Three-year survival was 87.7% in SAVR and 85.7% in TAVR.

Abbreviations: SAVR, surgical aortic valve replacement; TAVR, transcatheter aortic valve replacement.

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Table 11 The most recent review of SAVR and TAVR outcomes including the PARTNER trial randomised control trials 5-year outcomes.

Study	Year	Cohort	Outcomes
Zweng et al. [25]	2016	High risk SAVR and TAVR patients with propensity matched cohort	• Primary endpoints were 30-day mortality and 2-yr survival with secondary endpoints looking at readmission within 30-days, new AF, heart block requiring PPM, significant paravalvular leak (>mild AR), stroke, pneumonia, and blood transfusion requirements [15]. • Survival at 2 yrs was 74% for TAVR and 80% in SAVR (in propensity matched pairs which yielded 44 pairs). • In the propensity matched analysis, 30-day mortality was 5% in both groups, requirement of PPM was higher in TAVR at 23% and 5% in SAVR, postoperative AF was higher in SAVR at 41% and 2% in TAVR. • The rates of paravalvular leak were 7% in TAVR and 0% in SAVR. Lack of statistical significance in the leak rate is likely due to lack of statistical power. • TAVR patients included were of high operative risk and no validated frailty score was used to guide treatment allocation.
Makkar et al. [26]	2020	5-yr outcomes for PARTNER 2 investigators of SAVR and TAVR patients Funded by Edwards Lifesciences	• At 5 yrs, death from any cause or disabling stroke was 47.9% in the TAVR group and 43.4% in the SAVR group. In the transfemoral access group, this was 44.5% and 42% respectively. In the transthoracic access group, this was 59.3% in the TAVR group and 48.3% in the SAVR group. In the overall population the incidence of death from any cause was 46% in the TAVR group and 42.1% in the SAVR group (56.9% in the transthoracic group for TAVR and 47.3% in the SAVR group). • Rehospitalisation at 5 yrs was higher in TAVR with 33.3% versus 25.2% for SAVR. Aortic valve intervention was higher in TAVR with 3.2% vs 0.8% in SAVR (10/21 cases due to progressive stenosis and 11/21 due to worsening aortic regurgitation in the TAVR group). • The postoperative aortic insufficiency was graded as mild or greater in comparing both groups. In independent analysis, at 5 yrs mild paravalvular leak was seen in 17% of TAVR patients and 3.5% of SAVR patients. At 5 yrs, moderate or severe paravalvular leak was seen in 4.1% of TAVR patients and 0.2% of SAVR patients. • The main findings were that there was no significant difference in primary endpoints of death from any cause or disabling stroke at 5 yrs; but TAVR was associated with higher incidences of mild, moderate, and severe paravalvular regurgitation, and valve related intervention and rehospitalisation was higher in the TAVR group vs SAVR.

Abbreviations: SAVR, surgical aortic valve replacement; TAVR, transcatheter aortic valve replacement; AF, atrial fibrillation; PPM, permanent pacemaker; AR, atrial regurgitation; PARTNER, Placement of AoRTic TraNscathetER Valves.

however in recent years in these registries have been resolved and align with North American data.

Due to a divide between ANZSCTS and Transcatheter Aortic Valve Implantation Registry (TAVI-ACOR) databases in TAVR data throughout Australia, a limitation in this analysis was not capturing all TAVR cases submitted into the TAVI-ACOR registry; however, a recent analysis of TAVR cases obtained from the TAVI-ACOR registry in 2019 only showed 865 in hospital cases collected up to this period (less than our 1,194) [39]. Therefore, this analysis captured a

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significant proportion of all documented TAVR procedures over the period utilised in our data collection. Further limitations in the interpretation of the ANZSCTS national database is the short-term (30-day) postoperative data that is collected. From the trends in the literature since TAVR was introduced, real world information concerning its value and clinical application results from long-term analysis of outcomes and complications which are starting to appear [26]. There have been multiple RCTs [5,9,10] and subsequent long-term studies [26] which reveal progressively worsening valvular incompetence, a rate of CHB and PPM insertion, and vascular complications, including aortic dissection, that are higher in the TAVR group versus SAVR group, and these findings have been replicated in our database analysis.

It should be acknowledged that that preoperative demographics of patients in this observational study have different comorbidities which may influence the results. This is a limitation of any observational study and is likely due to, and may be influenced by, selection bias. We attempted to address this by using propensity score matching although we were unable to able to identify a sufficient number of matches to perform a meaningful analysis. Nevertheless, the difference in outcomes appear to be related more to the unique technical challenges associated with each procedure.

Conclusions

With an understanding of limitations in TAVR ANZSCTS data to date, this database analysis was deemed to have an insufficient number of participants for analysis and comparison between groups. Despite these recognised limitations, SAVR and TAVR outcomes over an 18-year period showed good primary endpoint results in mortality and permanent stroke across both groups, and readmission for surgical or valvular complications. Areas of difference remain in the degree of complete heart block and resulting need for PPM insertion, and vascular complications, including limb ischaemia and dissection. Although primary and secondary endpoints have remained similar across the two groups, secondary complications are severe and life threatening, and have shown to be significant in this analysis. There would be value in a combined or linked ANZSCTS and ACOR-TAVI database to capture the outcomes of these complications and perform complex analyses that carry high morbidity and mortality in the short and long term.

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RESEARCH ARTICLE Open Access

Quality of life and frailty outcomes following surgical and transcatheter aortic valve replacement

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Abstract

Background: Our objective was to report on the prospective outcomes in the areas of depression, quality of life, angina, and frailty in SAVR and TAVR patients with aortic stenosis undergoing aortic valve intervention.

Methods: We recruited 300 patients across 3 groups (TAVR, SAVR, and CABG) over 12 months. Depression, quality of life, frailty, and angina were assessed followed by propensity score matching.

Results: Using logistical regression when all patient factors considered for all patients who had SAVR and TAVR, the only preoperative factors that impacted on 1 year mortality was hypertension and STS score. Quality of life improvements within each group over 12 months was significant (p value = 0.0001). Depression at 12 months between groups (p value = 0.0395) and within each group was significant (p value = 0.0073 for SAVR and 0.0001 for TAVR). Angina was most frequent in TAVR at 12 months in the QL (p = 0.0001), PL (p = 0.0007), and improvement was significant in the QL (SAVR p = 0.0010, TAVR p = 0.0001) and PL (SAVR p = 0.0002), TAVR p = 0.0007) domains in both groups. Frailty at 12 months improved in both groups, but was greatest in TAVR (p value = 0.00126).

Conclusions: This 12 months follow up of cardiac surgical patients has revealed significant improvement in PROMs and frailty in all groups by 3 months postoperative regardless of surgical or transcatheter approach. Outcome measures of quality of life and frailty could be utilized as a measure of outcome more regularly in patients undergoing aortic valve surgery regardless of approach.

Keywords: Quality of life, Frailty, Depression, Angina, PROMS

Background

Aortic valve replacement is designed to prolong life and improve its quality, with the latter being particularly relevant given the elderly patient's undergoing this procedure. The early studies reporting on quality-of-life analysis in aortic valve surgery patients were first published in 1997 [1–3]. PROMS were first applied in the areas of heart failure [4] and later to heart valve surgery in 2016 [5]. And determined the value in assessing a patient's quality of life before and after cardiac surgery.

Our primary endpoint is to determine quality of life between SAVR and TAVR in aortic stenosis (including CABG as a control) over a 12 months period. Our secondary aims are to determine and compare the angina, depression, and frailty outcomes between these groups. We hope that this information will help guide preoperative, perioperative, and postoperative management of

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patients undergoing aortic valve replacement in these crucial domains that determine patient satisfaction post aortic valve intervention.

Methods

Patient recruitment

Following ethics and governance approval (CALHN) (HREC/18/CALHN/188), between June 2018 and August 2020, a total of 300 patients across 3 groups were recruited consecutively from a single institution, at the Royal Adelaide Hospital, Adelaide, South Australia. The 104 three groups comprised a SAVR (100 patients), TAVR (100 patients) and coronary artery bypass grafting (CABG) group (100 patients). All patients were contacted directly, and consent obtained to participate in this data collection that would occur over a 12 months period. Inclusion criteria was patients undergoing a single cardiac procedure (SAVR, TAVR, CABG only) without associated coronary intervention (PCI). Patients excluded had combined procedures, a major perioperative complication precluding continued involvement, patients who died, or who declined involvement. Those patients who declined involvement were replaced with a newly recruited patient to reach the prespecified sample size.

Baseline demographics

Socio-demographic, symptoms, comorbidities, and risk factors were collected at baseline from the patients as well as hospital records as presented in Table 1.

Table 1 Baseline demographics, comorbidities, and cardiac function obtained from the study cohort

Average baseline characteristic	SAVR n = 100	TAVR n = 100	CABG n = 100
Mean age	65.94 (SD 11.6)	82.87 (SD 6.9)	65.90 (SD 10.0)
Gender (male)	79/100	80/100	79/100
Diabetes mellitus	19/100	38/100	46/100
Hypertension	56/100	69/100	74/100
Previous stroke/TIA	5/100	6/100	11/100
AF	10/100	32/100	6/100
eGFR < 90 ml/min	1/100	26/100	8/100
Pulmonary HTN	2/100	2/100	0/100
COPD	13/100	12/100	14/100
Existing PPM	1/100	13/100	0/100
PVD	1/100	10/100	2/100
NYHA class	2.23 (SD 0.7)	2.61 (SD 0.6)	1.13 (SD 0.4)
LVEF	57.95 (SD 8.4)	54.62 (SD 11.8)	54.33 (SD 11.0)
AVA cm^2	0.99 (SD 0.5)	0.82 (SD 0.3)	2.67 (SD 0.7)
Mean AV gradient	46.57 (SD 15.4)	41.10 (SD 13.8)	5.71 (SD 4.9)
History of CAD	11/100	49/100	100/100
STS score (%)	1.18 (SD 0.4)	4.82 (SD 3.0)	0.77 (SD 0.4)
Cohort mortality	2/100	7/100	0/100

TIA transient ischemic attack, *eGFR* estimated glomerular filtration rate, *HTN* hypertension, *COPD* chronic obstructive pulmonary disease, *PVD* peripheral vascular disease, *NYHA* New York Heart Association, *LVEF* left ventricular ejection fraction, *AVA* aortic valve area, *CAD* coronary artery disease, *STS* society of thoracic surgeons

Health status instruments

Depression was measured using the Patient health questionnaire 9 (PHQ-9) [5–9]. There are 9 domains in the questionnaire with a score assigned 0–3 (0 being no depressive thoughts and 3 being depressive thoughts nearly every day). A range of scores from 0 to 27 are possible. Scores of 5, 10, 15, and 20 represent cut points for mild, moderate, moderately severe, and severe depression, respectively [10].

Quality of life was measured using the Euro QOL EQ-5D questionnaire [5, 11–16]. Quality of life scores were separated into 5 domains with a score of 1–3 giving the patient health profile [17]. A health state score of 1 indicates no problems, a score of 2 indicates some problems, and a score of 3 indicates extreme problems.

Frailty was measured using the Essential Frailty Toolset (EFT) which is a 4-item screening tool incorporating a chair rise activity which is self-reported, any cognitive decline which is reporter assessed, haemoglobin level, and serum albumin level. A score of 3 points indicates frailty [18, 19], while a higher score of > 4 was associated with a reduced 2 years survival [19], and others associated higher all-cause mortality at 1, 2, and 3 years with higher modified EFT scores [20].

Angina was measured using the Seattle Angina Questionnaire (SAQ-7). The SAQ7 consists of 7 questions that reports on activities performed over a 4 weeks period and any specific limitations or symptoms of angina that have impacted on the patient in this time. A score 128 of 0–35 is assigned with 0 indicating the most limitation, pain, and impact on the patient's quality of life. Three domain scores and one summary score are generated from the SAQ-7 [21].

- A Physical limitation score (SAQ7-PL). The Physical limitation score assesses the degree of physical limitation over the past 4 weeks due to various activities representing mild, moderate, and severe exertion.
- An Angina frequency score (SAQ7-AF). The Angina frequency score assesses the frequency of angina symptoms over the past 4 weeks with higher scores representing lesser angina burden.
- A Quality-of-life score (SAQ7-QL). The Quality-of-life score assesses how the patient perceives their CAD to be impacting his or her QOL.

- A SAQ7 summary score. The SAQ summary score assesses the average of SAQ-PL, SAQ-AF, and SAQ QL scores [21].

Data collection

Questionnaire data was collected at five independent time periods as inpatient or by telephone questionnaire during the 12 months. The time periods consecutively collected were preoperatively (within 4 weeks of procedure), postoperatively (prior to hospital discharge), 3 months postoperatively, 6 months postoperatively, and 12 months postoperatively.

Data was collected by two investigators over this period, with each investigator reviewing the questioning process and data collection to ensure interobserver reliability. Data analysis was completed by the primary investigator.

Statistical analysis

Power for recruitment sample size was calculation at 0.05 and 90% power accounting for a 10% dropout rate with 110 patients recruited to satisfy power. Statistical analysis was performed using GraphPad Prism 6 (GraphPad So 152 software, San Diego, California). A p-value of < 0.05 was considered significant.

An unequal variance t-test (Welch's t test) was used to compare SAVR and TAVR EQ5D health state, and 12 months EQ5D outcomes due to their equal means and normal distribution. A non-parametric test (Mann-Whitney U test) was used to compare EQ5D health score preop and at 12 months in SAVR and in TAVR due to differences in median and not-normally distributed independent groups. It was used to compare SAQ7 preoperative and 12 months scores between SAVR and TAVR, compare preoperative and 12 months scores in the SAVR group and independently in the TAVR group. This was performed in all subdomains of the SAQ7 test. It was used to compare preoperative and 12 months PHQ9 scores between SAVR and TAVR, preop and 12 months scores in the SAVR group and independently in the TAVR group. It was used to compare preoperative and 12 months EFT scores between SAVR and TAVR, and compare preoperative and 12 months scores in the SAVR group and independently in the TAVR group.

Logistical regression followed by propensity score matching was performed using SPSS. We performed a stepwise logistical regression analysis using all known patient preoperative demographics and co-morbidities that were collected. The dependent variable was 1 year mortality. Propensity matching was subsequently performed using the outcome of the logistical regression analysis with a tolerance of up to 1.

Results

A total of 331 patients were approached during the study to participate in the data collection process. A total of 31 patients declined to be involved for various reasons and subsequently were not included in the data analysis. No patients during the 12 months period declined to continue their involvement in the study, and no patient was lost to follow-up, however 9 patients died through the 12 months data collection period: 7 patients from the TAVR group and 2 patients from the SAVR group.

EQ-5D depression measurements

SAVR had the best quality of life regarding mobility (1.10) followed by TAVR and CABG respectively, $p=0.40$. In terms of self-care, CABG had the best quality of life (1.01), followed by SAVR and TAVR, $p=0.40$. In usual activities, CABG had the best quality of life (1.57) followed closely by SAVR (1.59) and TAVR, $p=0.02$ and 0.42 respectively. Pain and discomfort were best in the TAVR group (1.24) followed by SAVR and CABG, $p=0.04$ and 0.30. In terms of anxiety and depression symptoms, TAVR reported least symptoms (1.07), followed by CABG and SAVR, $p=0.02$ and $p=0.07$. The EQ-5D testing domains are summarized in Table 2 and Fig. 1.

Patient's own perspective of their health status over the 12 months period is summarized in Table 3. The best

Table 2 Domain measurements of EQ5D Quality of life in the 3 cohorts over 12-months analysis period

Domains	Mobility			Self-care			Usual activities			Pain/discomfort			Anxiety/depression		
	SAVR	TAVR	CABG	SAVR	TAVR	CABG	SAVR	TAVR	CABG	SAVR	TAVR	CABG	SAVR	TAVR	CABG
Preoperative	1.07	1.46	1.44	1.26	1.85	1.36	1.64	2.00	1.75	1.45	1.26	1.67	1.29	1.17	1.14
Postoperative	1.19	1.17	1.35	1.37	1.24	1.29	1.7	2.10	1.87	1.51	1.54	1.56	1.37	1.06	1.20
3 months	1.08	1.03	1.05	1.2	1.13	1.02	1.59	1.81	1.49	1.34	1.26	1.23	1.17	1.00	1.01
6 months	1.11	1.03	1.04	1.1	1.18	1.01	1.55	1.73	1.39	1.27	1.12	1.23	1.04	1.01	1.01
12 months	1.07	1.03	1.04	1.11	1.25	1.01	1.47	1.66	1.36	1.24	1.04	1.19	1.10	1.00	1.01

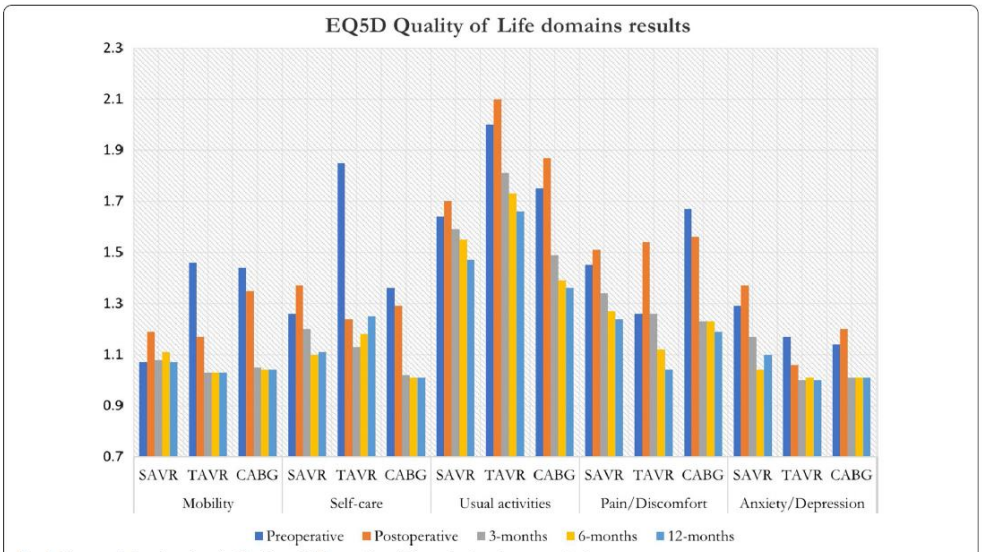

Fig. 1 Bar graph showing the distribution of QOL results within each domainamongst all groups

Table 3 Patient's own health score given over the 12 months period as a visual analogue scale (VAS) from 0 to 100. A score of 100 indicates the best health a patient perceives themselves to be in at the time

Cohort	SAVR	TAVR	CABG
Preoperative	63.30	57.80	59.50
Postoperative	63.90	70.51	64.00
3 months	72.20	74.44	76.45
6 months	73.50	74.80	79.45
12 months	75.40	75.97	79.65
Average VAS score	69.66	70.70	71.81
Median	72.2	74.4	76.5
IQR	63.6–74.5	64.2–75.4	61.8–79.6

health status score was in the CABG group at 12 months, followed by TAVR and then SAVR.

Patient results from their own perception of their health or the EQ5D Visual Analogue Scale (VAS) are shown in the Table 3 and Fig. 2.

Each patient's preoperative and 12 months health state was determined in the SAVR and TAVR groups. Preoperative health state between SAVR and TAVR using an un-paired t-test with Welch's correction showed a significant difference (p value = 0.02). At 12 months, the SAVR and TAVR groups mean values were the same, and following statistical analysis as above, there was no significant difference between the two (p value = 0.80). When comparing each group separately from preoperative to 12 months health state using the Mann–Whitney U test, SAVR showed a significant difference (p value < 0.0001), and TAVR showed a significant difference (p value < 0.0001).

PHQ-9 depression measurements

Preoperative depression analysis using Mann–Whitney U test showed significant difference between SAVR (2.31) and TAVR (2.54) (p value = 0.0142). SAVR (median 0.0, IQR 0 – 3); TAVR (median 2, IQR 0–4).

Postoperatively, the range was 0–13 in the CABG group, 0–13 in the TAVR group, and 0–16 in the SAVR group. At 3 months follow-up, depression scores ranged from 0 to 14 in the CABG group, 0-5 in the TAVR group, and 0–16 in the SAVR group. At 6 months follow-up depression scores ranged from 0 to 10 in the CABG group, 0–6 in the TAVR group and 0-15 in the SAVR group. At 12 months, depression scores ranged from 0 to 10 in the CABG group, 0–6 in the TAVR group, and 0–15 in the SAVR group. Postoperative depression analysis using Mann–Whitney U test showed significant difference between SAVR and TAVR (p value = 0.03).

No patients reported symptoms of suicidal or homicidal ideation throughout the questionnaire process.

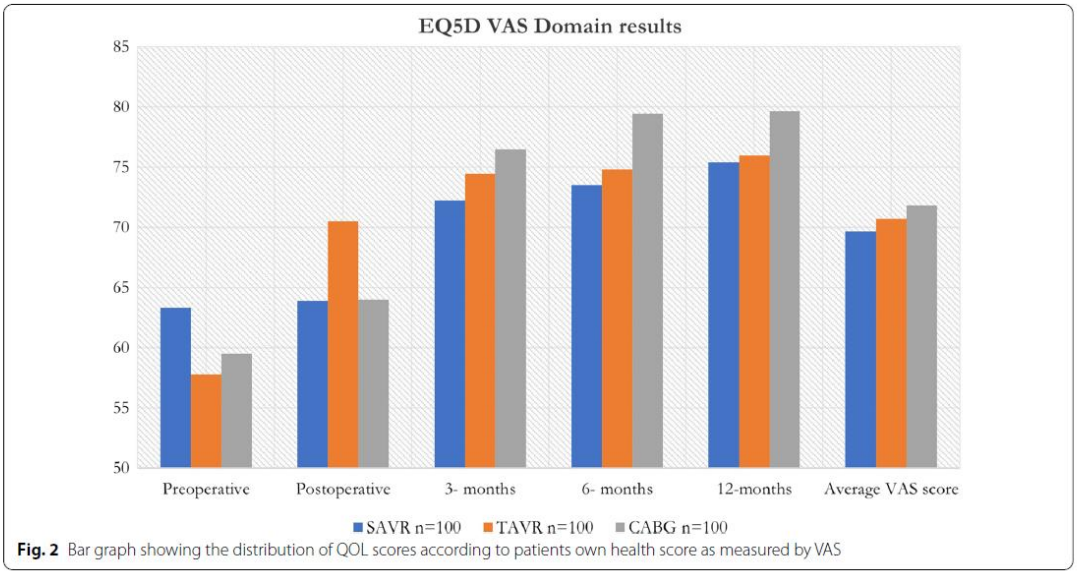

Fig. 2 Bar graph showing the distribution of QOL scores according to patients own health score as measured by VAS

Table 4 PHQ-9 measure of depression over a 12 months period across the 3 cohorts. A score of < 1 denotes no depressive symptoms and < 5 denotes minimal depressive symptoms

Cohort	SAVR n = 100	TAVR n = 100	CABG n = 100
Pre-operative	2.31	2.54	2.33
Postoperative	2.24	2.17	3.15
3 months	1.23	1.17	1.52
6 months	0.99	1.02	0.83
12 months	0.78	0.92	0.89
Average PHQ-9 score	1.51	1.56	1.74
Median PHQ-9 score	1.23	1.17	1.52
IQR	1.0–2.2	1.0–2.2	0.9–2.3

Those who scored higher on the symptom scoring, were referred accordingly. Average depression scores were low in all groups. The SAVR group had the lowest score (1.51) followed by TAVR (1.56) and CABG (1.74) respectively.

Intergroup analysis of preoperative and 12 months depression scores using Mann-Whitney U test showed statistically significant results in the SAVR (p value = 0.01) and TAVR (p value =0.0001).

Depression measurements as per the PHQ-9 questionnaire over the 12 months data collection period can be summarized in Table 4 and Fig. 3.

EFT frailty measurements

Frailty in the TAVR group was worse preoperatively compared to SAVR. Using the Mann–Whitney U test, this was significantly different (p value = 0.02).

Average frailty scores were higher in the TAVR group (0.98), and CABG group (0.97) compared to the SAVR group (0.83). Noticeably preoperative TAVR frailty scores were higher than the other cohorts (1.08). Only the CABG group in the postoperative measurements (3.15) reached a level of classification as frail. Statistically, the SAVR and TAVR differences at 12 months were not significant (p value = 0.07).

Intergroup analysis revealed no significant difference 225 in frailty over the 12 months in the SAVR group (p226 value = 0.05) and a significant difference in the TAVR group (p value = 0.01). Frailty measurements as per the EFT over the 12 months data collection period are summarized in Table 5 and Fig. 4.

SAQ-7 angina measurements

In the measurement of angina outcomes, preoperative scores in the physical limitation (SAQPL) were worse in the CABG group (88.13), followed by TAVR (91.53) and SAVR (94.87) respectively. The difference between SAVR and TAVR preoperatively was significantly different (p value = 0.0002). Scores in the angina frequency (SAQAF) were worse in the CABG group (84.66), and almost equal in the SAVR (99.58) and TAVR (99.91) groups. The difference between SAVR and TAVR preoperatively was

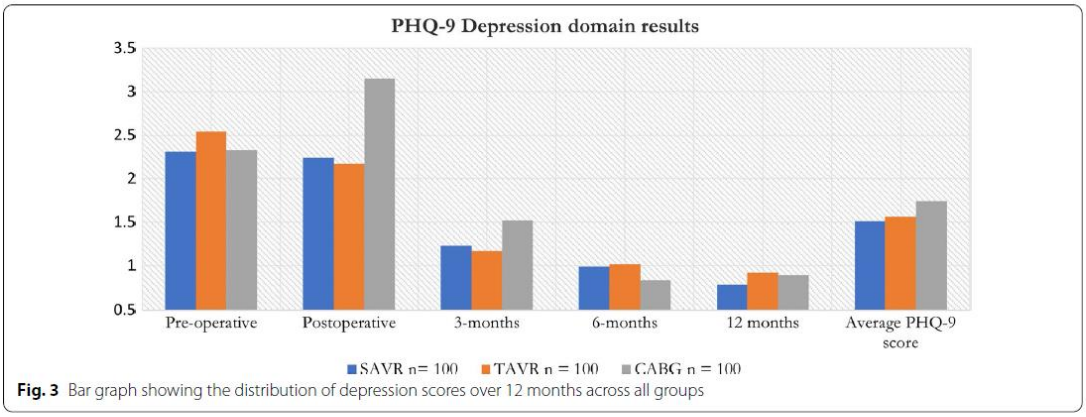

Fig. 3 Bar graph showing the distribution of depression scores over 12 months across all groups

Table 5 EFT measurements of frailty over a 12 months period. Scores of 3 or > were classified as frail

Cohort	SAVR n = 100	TAVR n = 100	CABG n = 100
Pre-operative	0.85	1.08	0.89
Postoperative	0.91	1.14	1.57
3 months	0.94	0.95	1.01
6 months	0.83	0.95	0.82
12 months	0.61	0.80	0.55
Average EFT score	0.83	0.98	0.97
Median EFT score	0.85	0.95	0.89
IQR	0.8–0.9	1.0–1.1	0.8–1.0

not significantly different (p value = 0.1213). Quality of life (SAQQL) was equal preoperatively between CABG (90.50) and TAVR (90.60) and lower in the SAVR group (94.50). The difference between SAVR and TAVR preoperatively was significantly different (p value < 0.0001). Summary scores across all subdomains indicated a higher angina score in the CABG group (87.76), followed by TAVR (94.01) and SAVR (96.32) respectively. The difference between SAVR and TAVR preoperatively was significantly different (p value = 0.0001).

Postoperative scores in the SAQPL group were worse in the TAVR group (91.80), followed by CABG (92.93)

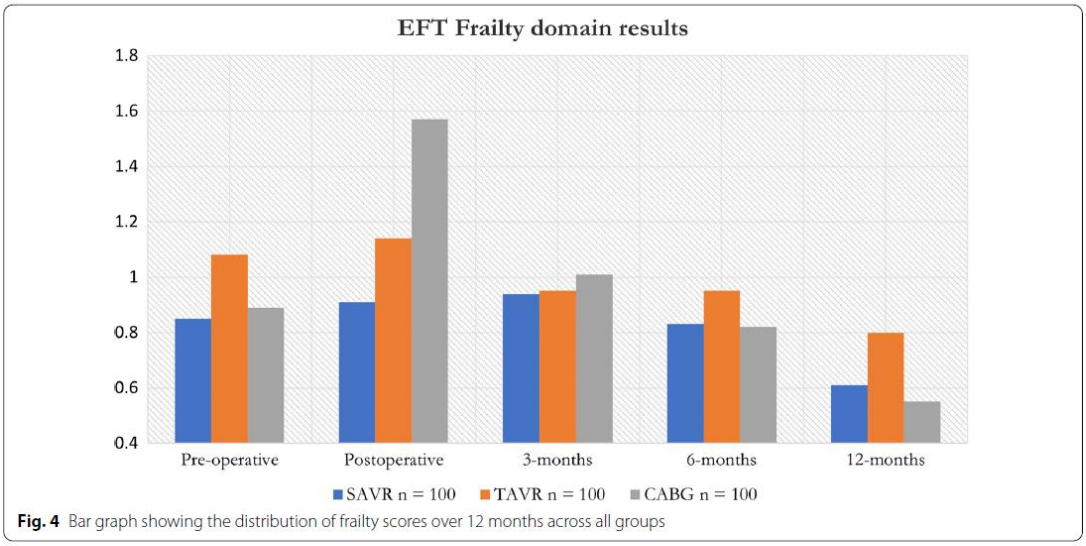

Fig. 4 Bar graph showing the distribution of frailty scores over 12 months across all groups

and SAVR (94.53). SAQAF scores were higher in the CABG group (90.42), with almost equal scores in the SAVR (99.58) and TAVR (99.91) groups. SAQQL scores were higher in the TAVR group (91.50), with equal scores in the CABG (94.00) and SAVR group (94.10). Postoperative summary score showed higher CABG scores (92.45), followed by TAVR (94.40) and SAVR (96.07).

Scores obtained at 3 months postoperatively in the SAQPL domain showed higher CABG scores (94.80), followed by TAVR (95.47) and SAVR (96.87). Scores in the SAQAF domain showed higher angina scores in the CABG group (95.75) and no reported anginal frequency in both the SAVR (100) and TAVR (100) groups. SAQQL scores were highest in the TAVR group (95.00), followed by almost equal scores in the CABG group (96.10) and SAVR groups (96.20). Summary scores showed higher scores in CABG (95.55) compared to TAVR (96.82) and SAVR (97.90).

Scores obtained at 6 months postoperatively in the SAQPL domain showed higher scores in the TAVR group (96.00), followed by the CABG group (97.33) and SAVR group (98.00). Scores in the SAQAF domain showed higher scores in the CABG group (97.83) with no reported anginal frequency at 6 months in the SAVR (100) and TAVR (100) groups. Scores in the SAQQL domain showed highest scores in the TAVR group (95.30), followed by SAVR (97.6) and CABG (98.30). Summary scores were highest in the TAVR group (97.10) followed by CABG (97.82) and SAVR (98.53).

Scores obtained at 12 months postoperatively in the SAQPL domain showed higher scores in the TAVR group (94.67), followed by CABG (97.33) and SAVR (98.40). The difference between SAVR and TAVR was significantly different (p value = 0.0007). Scores in the SAQAF domain were highest in the CABG group (97.83), followed by TAVR (99.58) and SAVR (100.00). The SAVR and TAVR 12 months scores were significantly different (p value = 0.0251). Scores in the SAQQL domain were highest in the TAVR group (95.80) followed by SAVR (98.10) and CABG (98.30). The 12 months SAQQL scores were significantly different between SAVR and TAVR groups (p value = 0.0001). Summary scores showed higher values in the TAVR group (96.68) followed by CABG (97.82) and SAVR (98.83).

Intergroup analysis showed a significant difference in the preoperative and 12 months SAQPL score in the SAVR group (p value = 0.0002) and TAVR group (p value = 0.0007). Intergroup analysis did not show a significant difference in SAQAF scores in the SAVR group (p value = 0.1213) but was significant in the TAVR group after 12 months (p value = 0.0251). Intergroup analysis showed a significant different in the SAQQL score for SAVR (p value = 0.0010) and TAVR (p value ≤ 0.0001).

Scoring of the subdomains in the SAQ7 questionnaire over the 12 months analysis period can be summarised in Table 6 and Fig. 5

SAVR versus TAVR we matched a total of 58 patients across both groups. Using logistical regression when all patient factors considered for all patients who had SAVR and TAVR, the only preoperative factors that had an impact on 1-year mortality was hypertension, and STS score (Table 7).

For the matched patients, we had a higher mean of 34.69 (SAVR) versus 34.07 (TAVR) for SAQ at 1 year which is statistically significant. The remaining results are not statistically significant but because of the low number of matched patients, a determination cannot be made (Table 8). Despite this, clinical significance of these outcomes and comparisons needs to be appreciated.

Discussion

In early registry data, [22] quality of life and frailty was extracted; however, the use of questionnaires was not included, including the PHQ-9, SAQ-7, and EFT. The Partner trials provided randomised outcomes between SAVR and TAVR [22–24], and reported that quality of life and health status were maintained at 12 months [23]. The Partner 2 trial in 2016 assessed baseline heath status using.

Table 6 Summary of the domain scores in the SAQ7 questionnaire including the patient SAQ Health score over the 12 months study period

Score	Preoperative			Postoperative			3 months			6 months			12 months		
	SAVR	TAVR	CABG	SAVR	TAVR	CABG	SAVR	TAVR	CABG	SAVR	TAVR	CABG	SAVR	TAVR	CABG
SAQ-PL	94.87	91.53	88.13	94.53	91.80	92.93	96.87	95.47	94.80	98.00	96.00	97.33	98.40	94.67	97.33
SAQ-AF	99.58	99.91	84.66	99.58	99.91	90.42	100.00	100.00	95.75	100.00	100.00	97.83	100.00	99.58	97.83
SAQ-QL	94.50	90.60	90.50	94.10	91.50	94.00	96.20	95.00	96.10	97.6	95.30	98.30	98.10	95.80	98.30
SAQ7 Summary score	96.32	94.01	87.76	96.07	94.40	92.45	97.90	96.82	95.55	98.53	97.10	97.82	98.83	96.68	97.82

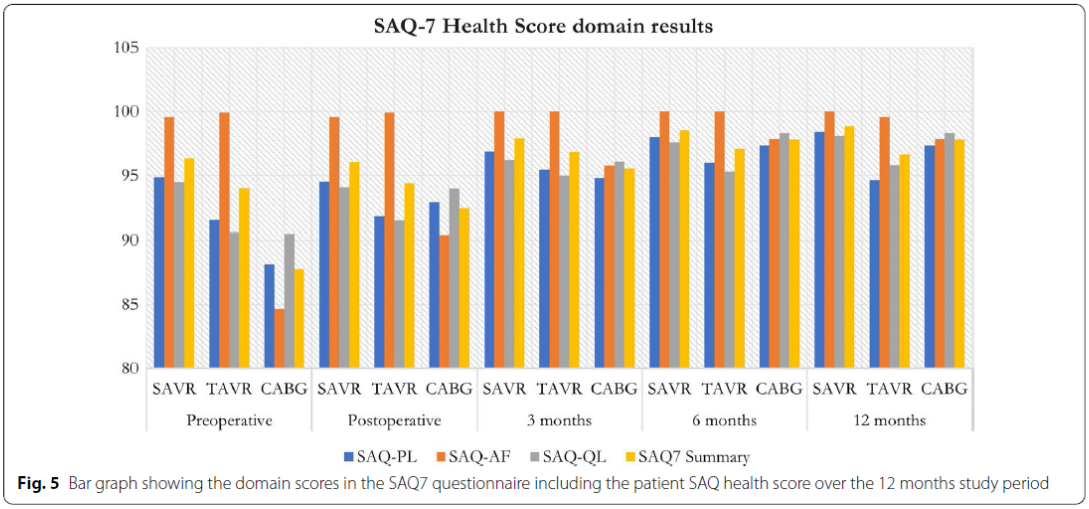

Fig. 5 Bar graph showing the domain scores in the SAQ7 questionnaire including the patient SAQ health score over the 12 months study period

Table 7 Preoperative variables influencing 1 year mortality outcomes across SAVR and TAVR groups following propensity score matching

Preoperative factor	p value
HTN	p = 0.0368
STS score	p = 0.0040

Table 8 Statistical analysis following propensity matching between SAVR and TAVR in all questionnaires

Questionnaire	Mean	p value
SAQ7	SAVR (34.69). TAVR (34.07)	p = 0.0429
PHQ9	SAVR (0.52), TAVR 0.63)	p = 0.6978
EQ5D	SAVR (7.55). TAVR (7.66)	p = 0.9530
EFT	SAVR (0.72), TAVR (0.96)	p = 0.3100

Kansas City Cardiomyopathy (KCCQ), SF 36, and EQ 5D questionnaires. This was reported over a 12 years follow-up [24]; and the Partner 3 trial in 2019 assessed functional status and quality of life at 30 days and 1 year using a 6-min walk distance, and (KCCQ) score. Conclusions were that TAVR had rapid improvements in symptoms of failure, 6 min walk distance [22]. Only the Partner 2 trial, used a specific quality of life questionnaire in the use of the EQ5D. In these large trials there has been less focus on quality of life and angina, and no reference towards depression and frailty as primary or secondary endpoints.

This prospective study determined that there is no significant difference in QOL between SAVR and TAVR over a 12 months period. It further went on to explore outcomes in the areas of depression, frailty, and angina in these groups as secondary endpoints.

It should be identified that TAVR patients were much older with more medical comorbidities, compared to the SAVR and CABG control group.

When we summarise the collective findings of the study we find that, quality of life outcomes were evenly distributed across the groups, depressive symptoms improved across all groups, and all groups including the TAVR group improved significantly in the measure of frailty at 12 months. Limited by power calculations and median similarities between median values, frailty results should be interpreted with caution.

Anginal scoring had the most complexity when it came to measurements of outcome. Compared to other instruments, the SAQ was the most responsive instrument to the anginal status and to the clinical change [25]. The SAQ was deemed more responsive than the SF-36 in terms of physical functioning when evaluating patients undergoing coronary bypass surgery (CABG) and angioplasty (PTCA) with a 3 months follow-up after revascularisation [26]. The improvement in physical limitation is noted in both SAVR and TAVR, while anginal improvement was highest in TAVR group compared to SAVR

In comparing the SAVR and TAVR groups, both remained free of significant anginal symptoms throughout the preadmission and postoperative follow-up. The TAVR group started at a higher risk and older age group and despite this had a steady improvement in physical

limitations, anginal frequency and quality of life over 12 months.

With the recognised importance in different presentations between men and women in ischemic heart disease [27–29]; measurements in quality of life could also be different in validated instruments. A retrospective multicentre analysis of over 10,000 patients including men and women showed comprehensive evidence that the SAQ is a valid patient-reported instrument that reliably helps capture the symptoms, functional status and quality of life related to angina, while also providing useful prognostic information in women with CAD [17].

In terms of study limitations, this is a prospective cohort study and inherently contains a selection bias, minimised with data collected consecutively. This is supported by a reduced number of propensity matched patients, likely related to lack of power because of reduced patient numbers and therefore reduced statistically relevant conclusions. The EFT has only been validated in the preoperative setting. All PROMS were conducted over the phone and by two investigators, whereas frailty measures were determined through the collection of 345 hospital data and over the phone in the measure of cognitive changes specifically. All three groups had different baselines and comorbidities. The data collection period was only over 12 months and will not capture the intermediate term complications. We recognize that the most important data will occur at least 7 years or more after a procedure when structural valve degeneration can have an impact. In an aim to reduce bias, propensity analysis via logistic regression analysis was performed.

Despite the limitations, the clinical value of such results should not be understated and we hope could supply value to the outcome measures. The SAQ for example is well-established in its validity, reproducibility, prognostic importance, and sensitivity to clinical change, but interpretation can be challenging because of lack of familiarity with the clinical importance of its domains, either cross-sectionally or longitudinally [30]. These questionnaires should be considered tools to support more patient-centred care, and a means of facilitating population health strategies to provide a better foundation for the integration of patient experiences with clinical care.

This study has shown that quality of life, depression, frailty, and angina improves across all groups of varied preoperative risk undergoing interventional and open cardiac surgical procedures over a 12 months period. Clinical evidence supports improvements across all domains and outcome measures for patients who undertake either SAVR or TAVR.

Following aortic valve surgery and coronary bypass surgery, symptoms impacting on a patient's quality of life reduce by 3 months postoperatively and improve to a point greater than their baseline functioning prior to their surgery regardless of pre-existing age and risk stratification. If we focus on optimising these areas, we may enhance a patient's perioperative quality of life when undergoing cardiac interventional and open surgical procedures.

Abbreviations

RCT: Randomised control trial; AR: Aortic regurgitation; AF: Atrial fibrillation; KPI: Key performance indicator; PROM: Patient related outcome measure; CABG: Coronary artery bypass grafting; PCI: Percutaneous coronary intervention; eGFR: Estimated glomerular filtration rate; TIA: Transient ischemic attack; PVD: Peripheral vascular disease; COPD: Chronic obstructive pulmonary disease; HTN: Hypertension; AVA: Aortic valve area; STS: Society of thoracic surgery; QOL: Quality of life; EFT: Essential frailty toolset; SAQ: Seattle angina questionnaire; VAS: Visual analogue scale; PTCA: Percutaneous transluminal coronary angioplasty; KCCQ: Kansa City cardiomyopathy questionnaire; ATSI: Aboriginal or Torres Strait Islander; BMI: Body mass index; BSA: Body surface area; EoA: Effective orifice area; PPM: Permanent pacemaker; NYHA: New York Heat Association; LVEF: Left ventricular ejection fraction; MI: Myocardial infarction; CCF: Congestive cardiac failure; IE: Infective endocarditis; CAD: Coronary artery disease.

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The authors had the freedom of investigation and full control of the design of the study, methods used, outcome parameters and results, analysis of data, and production of the written report.

Author contributions

TLS was the primary investigator involved in all data collection, analysis and author of the manuscript. JMA was involved in manuscript production and interpretation of data. JK was involved in data collection and patient questionnaires. HES was involved in manuscript editing and manuscript production. RRT, JMM, MW, JE, and JB were all involved in manuscript formulating and editing of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data incorporated into manuscript.

Declarations

Ethics approval and consent to participate

Ethics and governance approval (CALHN) (HREC/18/CALHN/188). All patients completed written and verbal consent forms and were provided patient information sheets and study outlines.

Consent for publication

All authors have approved this paper for publication without any bias.

Competing interests

Nil competing interests from listed authors.

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