Delineation of Ascending Aortic Medial Degeneration using Diffusion Tensor Imaging

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Medical Biophysics
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ABSTRACT AND KEYWORDS

A thoracic aortic aneurysm (TAA) is a life-threatening dilation of the thoracic aorta that can lead to catastrophic dissection or rupture. In TAAs, the structure of the aortic wall is perturbed. Current imaging approaches assess lumen dimensions but lack the ability to delineate aortic wall architecture, a key determinant of aortic integrity. Diffusion tensor imaging (DTI) is an MRI modality which can reveal tissue microstructure through diffusion characteristics. In this thesis I investigated the potential for DTI to evaluate TAA architecture. An ex vivo porcine model of aortic wall degeneration was developed, and DTI scans were performed on healthy and degenerated porcine ascending aortas. The findings revealed that, DTI scalar indices correlated with histological markers of aortic damage. Subsequently, ex vivo DTI assessment of human ascending aortic aneurysms revealed that abnormalities in DTI scalar indices correlated with medial degeneration including regional cellularity and regional glycosaminoglycan deposition. Together, the findings provide a strategy for imaging the architecture of the diseased and degenerating thoracic aorta.

Keywords: Thoracic aortic aneurysm, thoracic aortic dissection, diffusion tensor imaging, glycosaminoglycan
SUMMARY FOR LAY AUDIENCE

The aorta is the largest artery in the body and is responsible for trafficking all the body’s blood as it is pumped out of the left ventricle of the heart. This vessel is subject to tremendous pressure and its wall must possess great tensile strength. To cope with this task, the aorta is highly organized, with around 40 layers of densely packed smooth muscle cells interwoven with elastin fibres and supportive collagen. However, the aorta can deteriorate and its function can fail. Strategies to image the aorta are critical. Currently, these strategies include echocardiography, computed tomography, and standard magnetic resonance imaging (MRI). These strategies can inform on the shape of the aorta, aortic diameter, and aortic wall thickness. However, these imaging methods lack the ability to assess the structural organization of the aortic wall itself. This limitation is an important gap, because the primary determinant of aortic structure and function is the integrity of the layered components of the wall. This thesis explored the potential of diffusion tensor imaging (DTI) as a strategy to characterize the internal organization of aortic wall elements. DTI is an MRI-based imaging method that tracks the movement of water and can be used to map fiber organizations in highly structured tissues such as white matter tracts in the brain. Here I investigate the ability of DTI to inform on aortic structural elements such as elastin fibre disruptions, smooth muscle cell density, and the presence of glycosaminoglycans. This analysis could set the stage for the application of DTI to evaluate the internal architecture of the aortic wall.
CO-CONTRIBUTORS

The work presented in Chapter 2 of this thesis is intended for adaptation and submission as an original research manuscript. As the primary author, I contributed to all aspects of this study: sample preparation, sample MRI, histology, as well as analyses. Additional contributions are as follows: H. Yin provided guidance on experimental design, injection assay development, and statistical analyses. E. Ho undertook the coding for 2D tensor angle analysis. C. O’Neil undertook sample preparation and histological staining.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>2D</td>
<td>Two dimensional</td>
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<tr>
<td>3D</td>
<td>Three dimensional</td>
</tr>
<tr>
<td>ACCF</td>
<td>American College of Cardiology Foundation</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
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<tr>
<td>B₀</td>
<td>External magnetic field</td>
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<tr>
<td>B₁</td>
<td>Radiofrequency field</td>
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<tr>
<td>BAV</td>
<td>Bicuspid aortic valve</td>
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<tr>
<td>°C</td>
<td>Degrees Celsius</td>
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<tr>
<td>CCS</td>
<td>Canadian Cardiovascular Society</td>
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<tr>
<td>CE-MRA</td>
<td>Contrast-enhanced MR angiography</td>
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<tr>
<td>cm</td>
<td>Centimeter</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
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<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
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<tr>
<td>ECG</td>
<td>Electrocardiograph</td>
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<tr>
<td>FSL</td>
<td>fMRI Software Library</td>
</tr>
<tr>
<td>FA</td>
<td>Fractional anisotropy</td>
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<tr>
<td>G</td>
<td>Gradient strength</td>
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<tr>
<td>GAG</td>
<td>Glycosaminoglycan</td>
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<tr>
<td>IRAD</td>
<td>International registry of acute aortic dissections</td>
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<tr>
<td>LDS</td>
<td>Loeys-Dietz syndrome</td>
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<tr>
<td>MD</td>
<td>Mean diffusivity</td>
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<td>MDCT</td>
<td>Multidetector computed tomography</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>MFS</td>
<td>Marfan syndrome</td>
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<td>Millimeter</td>
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<td>Magnetic resonance</td>
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<td>RD</td>
<td>Radial diffusivity</td>
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<td>RF</td>
<td>Radiofrequency</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
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<td>s</td>
<td>Second</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SMC</td>
<td>Smooth muscle cell</td>
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<td>SSFP</td>
<td>Steady state free procession</td>
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<td>Tesla</td>
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<tr>
<td>T1</td>
<td>Spin-lattice relaxation time (longitudinal)</td>
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<td>T2</td>
<td>Spin-spin relaxation time (transverse)</td>
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<tr>
<td>TAA</td>
<td>Thoracic aortic aneurysm</td>
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<tr>
<td>TAD</td>
<td>Thoracic aortic dissection</td>
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<tr>
<td>TE</td>
<td>Time-to-echo</td>
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<tr>
<td>TEE</td>
<td>Transesophageal echocardiography</td>
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<tr>
<td>TGF-β</td>
<td>Transforming growth factor beta</td>
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<tr>
<td>TR</td>
<td>Time-to-repetition</td>
</tr>
<tr>
<td>TTE</td>
<td>Transthoracic echocardiography</td>
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CHAPTER 1

1 INTRODUCTION

1.1 General Overview

A key determinant of cardiovascular health is the vast and complex network of blood vessels tasked with transporting blood to all tissues in the body. The thoracic ascending aorta is critical in this regard. It is the largest blood vessel in the body, and it connects all oxygenated blood, pumped from the heart to the downstream vasculature. The ascending aorta possesses considerable tensile strength in order to withstand the unique biomechanical stresses associated with receiving blood pumped from the heart.\(^1\)

However, the ascending aorta is susceptible to disease and functional failure. Thoracic aortic aneurysms (TAAs) are one of the most prevalent types of thoracic aortic disease. Aneurysms are the pathologic and progressive dilatation of a blood vessel. If left untreated, TAAs, can dilate to the extent where the aorta is at risk of dissection or rupture; both potentially fatal complications even if promptly addressed.

TAAs are becoming increasingly prevalent in the general population. The incidence of thoracic aortic disease rose by 52\% in men and by 28\% in women, to reach 16.3 per 100 000 population per year and 9.1 per 100 000 per year, respectively, from 1987 to 2002.\(^2\) Importantly, TAAs develop asymptptomatically. Prior to acute events, 95\% of TAAs are asymptomatic.\(^3\) Mortality associated with this disease is very high. Risk of
mortality is estimated to increase as much as 1% per hour in the early phase after the onset of complications-related symptoms.\textsuperscript{4} Despite the relatively low incidence per capita, the consequences of this ailment are extremely serious, and potentially fatal. Unlike its better-characterized counterpart, abdominal aortic aneurysms, whose progression can be correlated heavily with atherosclerotic markers, TAAs are more prevalent and have far worse outcomes.\textsuperscript{5}

Given the severity of complications associated with TAAs, as well as the risks associated with surgical intervention, there is a need for precise diagnostic methods to assess the progression of TAAs. Accurate assessment of the extent of aortic disease can inform clinical decision making. The current gold standard for the assessment of TAAs is a thoracic CT-scan, MRI, or echocardiogram. These diagnostic scans will provide information regarding aortic cross-sectional morphology and diameter. Due to variability in aortic thickness, inflammation, and morphology, the only imaging-derived metric used by physicians in clinical assessments is the aortic diameter.

Clinicians will assess a surgical intervention threshold for each patient, most commonly 5.5 cm in luminal diameter. This triggers the decision to replace the dilated aortic segment with a graft. However, evaluation of data from the International Registry of Acute Aortic Dissections (IRAD) has indicated that a diameter intervention threshold of 5.5 cm is not a reliable intervention “cut-point” in preventing the occurrence of ascending aortic dissections.\textsuperscript{6} In fact, dissection frequency histograms based on ascending aortic diameter reveal a broad non-standard distribution.\textsuperscript{7} A significant proportion of
dissections occurring at smaller aortic diameters. Surgical intervention could be undertaken at smaller aortic diameters to potentially prevent dissections that are missed by the current intervention guidelines. However, this approach would lead to unnecessary prophylactic aortic replacements in many patients, exposing them to unnecessary surgical risks. The distribution of dissection diameters indicates that extensive dilation is not necessarily a precursor for dissection. The use of population-based studies or family history may help in the estimation of complication risks, but these are not a precise nor a patient-specific measure. As a result, there is a need for diagnostic imaging that can provide further direct insights, outside of diameter and demographic assessments, into the risk of adverse aortic events in individuals with TAAs. The underlying cause of thoracic aortic aneurysm and dissection is the degree of tissue damage in the aortic wall.\textsuperscript{8–10} A non-invasive imaging modality that could provide insights into aortic wall integrity has the potential to vastly improve TAA risk assessment.

Diffusion tensor imaging (DTI) is an MRI modality capable of assessing tissue microstructure characteristics through the analysis of water diffusion patterns within the tissue. DTI has seen frequent application in the brain as well as some preliminary studies in the heart. As a first step toward the clinical application of DTI in the characterization of the aortic wall, the goal of this thesis was to determine if DTI could detect disease-related changes in aortic wall composition \textit{ex vivo}. To address this goal, I have pursued three specific objectives:

1. To characterise the diffusion characteristics of healthy aortic tissue.
2. To develop a model of aortic wall degeneration, similar to human disease and subsequently evaluate this model using DTI.

3. To perform DTI scans on a human cohort of ascending aortas to confirm whether DTI was sensitive to varying degrees of aortic disease.

1.2 Aortic Structure and Function

The aorta is the largest artery in the body, delivering blood pumped from the heart to all downstream blood vessels. The aorta originates from the left ventricle at the aortic valve. The aortic sinus of Valsalva extends above the annulus of the aortic valve, expanding slightly before narrowing at the sinotubular junction. The aorta then continues upward forming the ascending aorta. The aorta subsequently forms the aortic arch. This arch gives off three branches: the brachiocephalic trunk, the left common carotid artery, and the left subclavian artery. These branches direct blood flow towards the brain and upper body. The aortic segment immediately downstream of the origin of the left subclavian artery is known as the aortic isthmus. The aorta then traverses downward towards the lower torso (Figure 1.1). This portion of the aorta is known as the descending thoracic aorta. Below the inferior border of the twelfth thoracic vertebra the aorta transitions to the abdominal aorta. My thesis focusses on the disease of the thoracic aorta. More specifically I focus on the ascending thoracic aorta, which is the region of the thoracic aorta commonly associated with aneurysms and dissections.
Figure 1.1 Diagram of the human aorta demonstrating its gross anatomy. Adapted with permission from Radiopaedia.org, rID: 56057

The aortic wall has 3 layers: the tunica intima, the tunica media, and the tunica adventitia (Figure 1.2). The tunica intima is the inner most layer. It is comprised of an endothelial cell monolayer, and a subendothelial space. The endothelial cells are flat, and
elongated, with their long axes parallel to the direction of blood flow. The subendothelial layer is occupied by loose connective tissue and interspersed smooth muscle cells, which typically increase in density as they approach the tunica media. The tunica media is a dense, highly ordered structure accounting for approximately 80% of aortic wall thickness. It is made up of smooth muscle cells, elastin lamellae, and collagen fibrils. These components are organized as circumferential layer. Most studies describe smooth muscle cell orientation as entirely circumferential, perpendicular to the direction of blood flow. However, others have reported smooth muscle cell orientation as primarily circumferential, but wrapping with a right-handed helix. Elastin lamellae and collagen fiber matrices form planar structures that wrap circumferentially and extend axially along the aortic wall, in the direction of blood flow. Elastin lamellae alternate with interlamellar smooth muscle cells, and collagen. The adult human tunica media contains 50-70 lamellar units. Each core of elastin lamellae is surrounded by microfibrils that extend into the adjacent interlamellar zone and form focal adhesions with interlamellar smooth muscle cells. This structural complex is known as a “lamellar unit” or “elastin-contractile unit” (Figure 1.3). Elastin microfibrils are composed of several glycoproteins, a prominent one being fibrillin (encoded by FBN1, FBN2, and FBN3). The interactions facilitated by microfibrils in the aorta are essential to the integrity of the aortic wall. Mutations in these genes are known to predispose individuals to thoracic aortic disease. Microfibrils are organized in an oblique orientation to the core elastin lamella and attach to dense plaques in smooth muscle cell membranes. Intracellular smooth muscle actomyosin is also connected to these membrane plaques.
Together these structures aid in smooth muscle cell mechanotransduction and long-term elastin stability.\textsuperscript{26,27} The tunica adventitia, is the outer-most layer of the aorta. It is comprised largely of loose collagenous tissue containing the vasa vasorum (microvessels) and nerves.\textsuperscript{25}

Figure 1.2 Full-aortic wall thickness photomicrograph of a Movat’s pentachrome-stained human ascending aorta. Labels depict the aortic wall layers.
The thoracic aorta performs a unique role in both conducting and regulating blood flow. The latter arises from its ability to act as an elastic buffering chamber in series with the heart, through a property known as the Windkessel function. During the systole the thoracic aorta contains approximately 50% of left ventricle stroke volume. Subsequently, during diastole, the aortic wall acts as an elastic buffer, forwarding a component of this stored volume. This buffering effect serves to reduce systolic blood pressure and enables nearly continuous peripheral blood flow.
1.3 Thoracic aortic aneurysm and dissection

1.3.1 Thoracic aortic aneurysm

The thoracic aorta is at its widest at its root. Aortic lumen diameter decreases distally. An average normal ascending aortic diameter is approximately 3.0 cm. A “normal” ascending aortic diameter depends on many factors. As individuals age, there is a progressive loss of aortic elasticity, and thus a tendency for diameter to naturally increase (Figure 1.4). Normal aortic diameter also increases with increased body surface area (Figure 1.5).
Figure 1.4 Ascending aortic diameter relationship to age. Mean normal ascending aortic diameter denoted by (♦). Upper limit for ascending aortic diameter as a function of age denoted by (▲). Reproduced with permission from Hannuksela et al. 31
Figure 1.5 The 95% normal confidence limits for the proximal ascending aortic diameter in relation to body surface area in adults <40 years of age. Reproduced with permission from Roman et al.32

An important disease associated with the thoracic aorta is thoracic aortic aneurysm (TAA). An aneurysm is a sustained enlargement of the lumen due to vessel wall expansion. The most common region of the aorta to become aneurysmal is the ascending aorta. An ascending aorta exceeding 150% of its expected normal diameter, is classified as an ascending thoracic aortic aneurysm.30,33 It is estimated that TAAs occur anywhere from 6 to 16.3 per 100,000 person-years.2,34 The overall prevalence of TAAs has steadily increased; from 1986 to 2002 the incidence of TAAs increased by 40% in the Swedish population.2 In the same time duration interventional operations increased 7-fold in men and 15-fold in women2.
TAAs were originally associated with a pathology termed “cystic medial degeneration”. However this term has been established to be incorrect as there are no cysts present in TAAs. However, several degenerative features are well established markers of TAA pathology. Table 1.1 summarizes these individual features, which are known to contribute to medial degeneration.

<table>
<thead>
<tr>
<th>Histopathologic Phenomenon</th>
<th>Description</th>
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| Glycosaminoglycan (GAG) extracellular matrix accumulation | • GAG: unbranched chains of disaccharide repeats including chondroitin/dermatan sulfate and hyaluronan.  
• GAGs interact and pool with each other to form large, water-retaining, gel-like aggregates.  
• GAGs may deposit into intralamellar space or form larger pools, spanning multiple lamellae and disrupt lamellar organization  
• Computational models show that GAGs fail to resist wall stress.  
• GAG pooling/swelling may cause medial delamination.  
• Visualized as blue patches in Movat’s pentachrome, trichrome, and Alcian blue staining |
| Elastin fiber fragmentation or loss          | • Loss or fragmentation of elastin lamellae  
• Visualized as disruptions or gaps in a lamella  
• May create foci or patches, with multiple layers lacking elastin lamellae  
• Visualized as disruptions in black elastin staining in Movat’s pentachrome |
| Elastin lamellar thinning                    | • Thinning of the usually dense layers of elastin fibers comprising each lamella.  
• Results in increasing interlamellar spacing and narrower, more frayed apart elastin fibers.  
• Visualized as a reduction in black elastin stain thickness in Movat’ pentachrome. |
| Elastic fiber disorganization | • Fibers are poorly organized  
  • Loss of typical organized, parallel structure.  
  • A rarer-occurring phenomenon.  
  • Visualized as poorly aligned, jagged black elastin staining in Movat pentachrome. |
|-----------------------------|----------------------------------------------------------------------------------|
| Smooth muscle cell nuclei loss | • Implies smooth muscle cell loss when visualized via H&E staining.\(^{38}\)  
  • Can occur in patches or in a band-like pattern  
  • Frequently accompanied by GAG accumulation |
| Smooth muscle cell (SMC) hyperplasia | • Increased synthesis of vascular SMCs due to excess mechanical strain, form of medial wall remodelling\(^ {42} \)  
  • Often found in areas of smooth muscle cell disorganization |
| Smooth muscle cell disorganization | • Non-parallel arrangement of smooth muscle cells of the media creating multifocal disarray of smooth muscle cells.\(^ {38} \)  
  • Frequently observed towards adventitial/intimal borders of the aortic media  
  • Abnormal nuclei morphology  
  • Irregular distribution of smooth muscle cells |

**Table 1.1 Description of unique histopathologic phenomena that occur in thoracic aortic disease**

These histopathologic phenomena can occur in different extents and in many unique combinations from case to case (Figure 1.6). This leads to complex and unpredictable patterns in TAA progression.
Figure 1.6 Organization of aortic components is disrupted in thoracic aortic aneurysms. A. Depicts a region of healthy aortic media stained with Movat’s Pentachrome. B. Depicts a diseased aortic media with SMC disarray, SMC loss, elastin dropout and GAG deposition, stained with Movat’s Pentachrome.

1.3.2 Thoracic aortic dissection

TAAs are considered the primary risk factor for thoracic aortic dissections (TADs). A dissection occurs when there is a tear in the intima and blood ruptures into media of the aortic wall. This promotes medial layer separation due to blood entering and tracking within the aortic wall (Figure 1.7). Aortic dissections are classified based on the area of the aorta in which they occur. Stanford type-A dissections involve the ascending thoracic aorta, and account for approximately two thirds of aortic dissections. 43 Dissections which occur beyond the brachiocephalic vessels are classified as Stanford type-B. Once dissection occurs, the area within the aortic wall that has separated and has allowed blood to enter, is known as the ‘false lumen’. After initial tear, the medial dissection and false lumen creation results in pronounced chest pain. If the dissection propagates along the medial false lumen, this can lead to the closing off of key branch vessels or rupturing into the pericardial sac around the heart. Furthermore, if the medial
tissue is slightly degenerated, the surging blood may not be contained within the media and can rupture, effectively tearing out of the aortic wall. All of these complications have a risk of mortality.\textsuperscript{2,4,13,43,44}

\textbf{Figure 1.7 Ascending aortic dissection mechanism.} Image shows tearing of intima and media resulting in separation of the medial layer and formation of a false lumen. Arrows indicate blood movement. Reproduced with permission from LeMaire and Russell\textsuperscript{4}

The incidence of TADs is estimated between 2.9 and 4.3 cases per 100,000 persons per year.\textsuperscript{4,43} This incidence also seems to be increasing over time. In the period of 1990-1994, the incidence of TAD was 1.5-fold higher than from 1980-1984.\textsuperscript{43} Moreover, mortality due to acute thoracic aortic dissections has historically been high. TAD is the 15\textsuperscript{th} leading cause of death in patients greater than 65 years of age.\textsuperscript{45} Thoracic aortic dissections necessitate surgical intervention. For unrepaired acute Stanford type-A TADs, mortality risk will increase by as much as 1\% per hour in the early phase after
symptom onset.\textsuperscript{4} The International Registry of Acute Aortic Dissections estimates a mortality rate of 20\% for those who do not seek medical intervention within the first 24 hours of symptom onset.\textsuperscript{46} Furthermore, an assessment of acute type A dissection outcomes via IRAD estimates an in-hospital mortality rate of 59\% for individuals treated medically and 23\% for those who undergo immediate surgical repair.\textsuperscript{47} Overall, there is a 30\% in-hospital mortality rate for individuals presenting with an acute Stanford type-A dissection.\textsuperscript{48}

1.4 Etiology of Thoracic Aortic Aneurysm

Based on the different underlying causes, thoracic aortic aneurysms can be categorized as one of the following: genetic (Marfan, Loeys-Dietz syndrome) non-syndromic genetic (familial), bicuspid aortic valve (BAV)-associated, and sporadic (degenerative).

1.4.1 Syndromic genetic aortic aneurysm

There are certain autosomal dominant gene mutations that can lead to TAA formation. Connective tissue disorders are a common cause of TAAs. These are diseases that affect the distribution and roles of collagen and elastin within the body. These disorders affect the aorta heavily since collagen and elastin are two of the main components within the aortic wall.

Marfan syndrome (MFS) is an autosomal dominant inherited connective tissue disorder most commonly caused by mutations in \textit{FBN1}, the gene encoding fibrillin 1.\textsuperscript{37,49}
Fibrillin 1 is a structural component of the extracellular matrix which is also involved in the regulation of transforming growth factor beta (TGF-β) availability.\(^5^0\) MFS manifests through multiple symptoms such as disproportionate limb lengths, nearsightedness, curved spine, and skeletal abnormalities. However, a key area of concern is the cardiovascular manifestations of this disease. The majority of MFS cardiovascular abnormalities manifest in the thoracic aorta. Thoracic aortic manifestations range from aortic stiffness to aortic aneurysm and dissection.\(^5^1\) Tissue histologic analyses demonstrate elastic lamellae fragmentation, excessive collagen, and the accumulation of GAGs.\(^5^2\) Aortic dilation may occur early in life in individuals with Marfan syndrome.\(^5^1\) Imaging via echocardiography is advised every 1 to 2 years with intermittent CT or MRI to visualize the entire thoracic aorta.\(^5^3\)

Loeys-Dietz Syndrome (LDS) is another autosomal-dominant connective tissue disorder. Genetically, LDS is caused by a mutation in one of a core set of genes (\(TGFBRI, TGFBRII, SMAD3,\) or \(TGFB2\)) which disrupts the TGFβ signalling pathway.\(^5^4,5^5\) In addition to skeletal (pectus excavatum, scoliosis), craniofacial (widely spaced eyes, cleft palate), gastrointestinal inflammation, and cutaneous manifestations (translucent skin, easy bruising), LDS patients are predisposed to widespread and aggressive arterial aneurysms.\(^5^4\) In contrast to MFS, LDS patients display a more aggressive, widespread set of vascular features. LDS patients are known to have arterial ruptures at early ages, with aortic dissections having been reported in LDS patients as young as 3 years of age.\(^5^6\) Initial reports of LDS cohorts revealed a mean age of death at
26.1 years of age.\textsuperscript{57} As with MFS, aortic screening must occur at frequent intervals, with aortic replacement surgery performed prophylactically to avoid aortic complications. Other known genetic conditions that are associated with TAAs include Turners syndrome, Ehlers-Danlos syndrome.

1.4.2 Non-syndromic genetic familial aortopathy

Mutation-related aortic medial degeneration is seen in individuals with or without a syndromic disorder. Consistent with this, TAAs occur more frequently in individuals with 1 or more relatives with thoracic aortic aneurysms and/or a previous history of aortic dissection. Family studies indicate that approximately 20\% of patients with TAAs have a first degree relative with the disease.\textsuperscript{58} This is referred to as “familial aortopathy”. There are many known and likely unknown heritable gene mutations causing a predisposition to TAAs. Non-syndromic mutations include: ACTA2, MYH11, MLCK, PRKG1.\textsuperscript{59} Data shows that TAAs progress quickly in familial aortopathy and surgical intervention is guided in part by the aortopathy history of other family members with this ailment.\textsuperscript{60}

1.4.3 Bicuspid aortic valve-associated TAA

A bicuspid aortic valve (BAV) is a structural abnormality that is associated with TAAs. BAV is one of the most common congenital heart defects; it affects 1-2\% of the population with a 2:1 male: female ratio.\textsuperscript{61} The aortic valve separates the left ventricle and the aorta. Cusps on this valve open and close with each heartbeat ensuring blood flows in the correct direction. In normal individuals, the aortic valve has three cusps
whereas individuals with BAV only have two cusps. Individuals with BAV are often unaware of their condition until later in life. In fact, BAV is most frequently asymptomatic and can cause minimal valvular dysfunction initially. However, cardiovascular complications such as aortic insufficiency, aortic stenosis, and endocarditis, occur largely after 50 years of age.\textsuperscript{62} Thus patients have regular assessments via echocardiography to monitor valve function as well assess aortic valve, root, and vessel diameters. Upon the onset of aortic valve regurgitation or stenosis, symptoms will progressively worsen necessitating surgical valve replacement.\textsuperscript{62} However, even after aortic valve replacement surgery, BAV patients are considered at-risk for TADs. An estimated half of identified-BAV patients demonstrate thoracic aortic dilatation.\textsuperscript{63} Furthermore, studies of BAV populations indicate the frequent presence medial degeneration, which suggests an aortopathy independent of abnormal valve function. In fact, it has been previously found that, of those undergoing aortic valve replacement surgery, 75\% had biopsy-proven evidence of medial degeneration in the ascending aorta.\textsuperscript{64} It is estimated that the risk of aortic dissection in patients with a bicuspid valve is 5 to 9 times higher than the general population.\textsuperscript{65}

### 1.4.4 Degenerative/Sporadic TAA

While there are many cases, where an underlying cause is identified to contribute to TAA formation, there are individuals who have no connective tissue disorders, no history of familial aortopathy, nor structural abnormalities, yet still develop a TAA. These individuals are typically older and have other cardiovascular risk factors such as
hypertension and history of smoking. Such cases are known as “degenerative” or “sporadic” TAAs. Despite not having a specific cause associated with a given TAA, this classification makes up the majority of TAA cases. It is estimated that degenerative TAAs encompass 60% of the total TAA incidence. Uncontrolled hypertension, and smoking are correlated with an increased risk for the development of TAAs.

1.5 Imaging of Thoracic Aortic Aneurysm

Individuals with a family history of genetic aortopathy, (Marfan, Loeys-Dietz, familial) typically undergo genetic screening for aortopathy-associated mutations as well as thoracic imaging at regular intervals to assess aortic dimensions. Outside of these populations, there are no regular screening strategies, and often no indication of disease prior to the emergence of severe complications. Degenerative and BAV-related TAAs are largely asymptomatic and commonly identified through imaging procedures for other purposes.

Once identified, a TAA will be initially imaged to determine aortic dimensions. Subsequently, clinicians will schedule interval-appropriate imaging sessions to monitor the progression of disease. Thoracic aortic imaging proceeds via one of three possible imaging modalities: echocardiography, computed tomography (CT), or magnetic resonance imaging (MRI). Each of these modalities have their own advantages and shortcomings.
1.5.1 Echocardiography

Echocardiography is a non-invasive imaging tool that is available at the bedside to visualize cardiac function. Two-dimensional images are produced via ultrasound, enabling visualization of myocardium, aorta and associated anatomical features. An ultrasound transducer will emit pulsed sound waves into the body. The sound waves will travel through the body until they contact the boundary of two different tissues. Here, some of the sound waves will be reflected back towards the transducer creating an “echo”. This echo can be interpreted as a one-dimensional image. A two-dimensional planar image can be generated by assessing a planar array from multiple echoes sent and received from a single ultrasound transducer. 68

The aorta can be assessed either by transthoracic echocardiography (TTE) or transesophageal echocardiography (TEE). TEE generally provides superior image quality and improved sensitivity than TTE. First, the use of higher frequency transducers is possible with TEE. Second, due to the close proximity to the transducer, TEE provides high quality images of the thoracic aorta. 69 Furthermore, given their early adoption into cardiac diagnostics, methods for cardiothoracic assessments via echocardiography are well established and serve as a reliable starting point. TEE has limitations. In some cases, images may not be acquired due to limited acoustical access, particularly in obese patients and in patients after surgery. 60 Accurate assessment of the aortic arch and proximal zones is often not possible. 70 Furthermore, for both TTE and TEE, assessment of aortic dimensions is subject to operator assessment error. 60
1.5.2 Computed tomography

ECG-gated, multidetector computed tomography (MDCT) is another, highly utilized imaging modality in the assessment of TAAs. Fast scanning, a wide field of view and high resolution enables detailed imaging. CT uses x-rays to build cross-sectional images. CT is based on the fundamental principle that the density of the tissue passed by the x-ray beam can be measured from the calculation of the attenuation coefficient. Using this principle, CT enables the reconstruction of body density in 2D slices perpendicular to the axis of acquisition. Multidetector CT improves upon conventional CT in two ways. First, MDCT has a high acquisition speed, approximately two times faster than conventional CT, such that, an entire acquisition can occur within the span of a single breath hold. Secondly, MDCT acquires volume data rather than slice data. These two factors together, enable MDCT to provide nearly 3D isotropic resolution data that can be arranged and viewed from different planes. The following are limitations regarding CT imaging of the thoracic aorta: CT scans expose individuals to prolonged x-rays, resulting in a relatively high dose of radiation for patients. Furthermore, contrast dyes place strain on the kidneys. Individuals with renal insufficiency may not be able to sufficiently filter dyes from their system.

1.5.3 MRI

MRI is a versatile imaging modality for thoracic aorta imaging. Using various targeted sequences, and multiplanar imaging, MRI can provide a detailed anatomical
view of the thoracic aorta. Various MR sequences are often used for measurement, including 3D contrast-enhanced MR angiography (3D CE-MRA), non-contrast 3D steady state free procession (3D SSFP), 2D cine SSFP, and black blood T2-weighted images. The most commonly used pulse sequence is 3D-CE MRA. Here, a gadolinium-based contrast material is injected into a peripheral vein. Image acquisition occurs when the contrast reaches the arterial system. A 3D T1-weighted sequence is used for acquisition, yielding good contrast between the thoracic aorta and veins in the background tissues. Images are acquired during breath holds and/or electrocardiograph (ECG) triggering.

MRI, similar to CT, has the advantage of producing high-resolution 3D anatomical images of the aorta. MRI offers flexibility for those who suffer from renal insufficiency. The most common MRI acquisition for the thoracic aorta (CE-MRA), makes use of contrast material. However, alternative acquisition sequences enable this shortcoming to be avoided, while still producing high-resolution cross-sectional aortic images. Furthermore, MRI is advantageous, in that it does not expose patients to ionizing radiation, as is the case with CT. Despite these advantages, MRI remains the least-utilized modality for thoracic aortic imaging, largely due to cost and lack of access. MRI scans are also associated with long wait times, especially in Canada. Furthermore, relative to MDCT scans, MRI suffers from longer acquisition times. Finally, this imaging modality is not available to those with paramagnetic surgical implants such as pacemakers.
1.5.4 Multi-modal thoracic aortic imaging outcomes

While the resolution, and image output varies significantly between echocardiography, CT, and MRI, these imaging modalities enable similar outcomes in regard to the diagnostic assessment of TAAs. For echocardiography, CT, and MRI, images are taken and measurements are made using a double oblique view of the ascending aorta. As such clinicians can monitor the precise site of aneurysm as well as aortic dimensions. While CT and MRI also allow visualizations of aortic morphology, both American Heart Association (AHA)/American College of Cardiology Foundation (ACCF) and Canadian Cardiovascular Society (CCS) recommendations state that abnormalities in aortic morphology should be recognized and reported separately when aortic diameters are within normal limits. Aortic diameter is the only quantitative metric extracted from current diagnostic imaging standards for the thoracic aorta which is a major limitation. No information regarding the presence of medial degeneration in the aortic wall, a key underlying factor in TAA development, can be extracted from currently utilized imaging modalities.

1.6 Interventions of Thoracic aortic aneurysm

1.6.1 Etiology-independent assessment framework

As previously mentioned, the progression of TAAs is largely asymptomatic until an acute catastrophic event. Medical management of TAAs is limited. Antihypertensive therapies may be employed to reduce the rate of aortic dilation. However, the main
intervention for TAAs remains surgical replacement. This surgery involves removing the damaged section of aorta and replacing it with a synthetic tube or graft. The outcomes of elective intervention on the thoracic aorta carry a much lower risk of mortality and morbidity than an acute aortic event. However, surgical intervention is highly invasive and does carry its own risks. Mortality from aortic replacement is estimated at 3-5%, and post surgical complications include irregular heartbeat, bleeding, stroke, graft infection and kidney damage.\textsuperscript{37,75} As such, within North America, clinicians follow intervention guidelines described by the CCS and ACCF/AHA to balance the risks of premature surgical interventions while minimizing the risks of acute aortic catastrophes.

After imaging assessments, clinicians will assess the progression of disease per aortic diameter measurements while taking into consideration patient specific factors. While the incidence of aortic complications increases as aortic diameter increases, the underlying nature of the TAA will play a role in its progression. As such, clinicians will consider patient-specific factors when determining the interval of follow-up imaging sessions and surgical cut-points. Factors such as age, and body surface area are positively correlated with aortic diameter. Additionally, the normal thoracic aorta is smaller in female and shorter individuals. Considering these factors allows the assessment of the extent of dilation outside of an expected normal aortic diameter for a given individual. Figure 1.5 shows the normal expected range of aortic diameter given age, and body surface area. It is estimated that normal aortic diameter increases 0.12 to 0.29 mm/y in a study evaluating 41 men and 36 women aged 18 to 82.\textsuperscript{53,76} Furthermore, it is estimated
that aortic diameter increases by 0.27 per unit of body mass index. Renal dysfunction, previous cardiac surgery, advanced age, smoking history, and uncontrolled hypertension are all considered risk factors for increased rates of aortic dilation.

Additionally, it is essential to consider the etiology of a given TAA. The rate of progression and expected complication diameters are influenced by the cause of the TAA as well as patient-specific factors.

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Details</th>
<th>CCS/ACCF recommended surgical cut-point</th>
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| Degenerative/Sporadic | • Most common etiology (10.4 cases/100,000 person-years).
• Associated with hypertension/aging.
• Acute complications increase significantly at 6.0 cm diameter.
• Average growth rate = 0.1 cm/yr. | 5.5 cm⁵³,⁶⁰ |
| BAV             | • Elevated expansion rate (0.2cm/yr).
• Often accompanies BAV treatment.
• 25% of patients require treatment for both TAA and BAV.
• If TAA diameter exceeds 4.0 cm, elective repair may accompany valve surgery. | 5.0 cm⁵³,⁶⁰,⁸²,⁸³ |
| Marfan          | • Rate of aortic expansion: 2.0-3.0 cm/yr.
• Complications occur at younger ages.
• 90-95% of complications occur at aortic diameter >5.0 cm. | 5.0 cm⁵³,⁶⁰ |
• Prophylactic repair at smaller diameters when rate of expansion exceeds 5.0cm/yr.\textsuperscript{53,60}
• Prone to multiple aneurysms, thus requiring regular imaging post surgical repair.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Details</th>
<th>Diameter</th>
</tr>
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| Loeys-Dietz | • Very early onset aortopathy.\textsuperscript{55}  
• Mean age of death 26 years.\textsuperscript{57} | 4.2 cm\textsuperscript{53,60} |
| Non-Syndromic Familial | • More rapid progression than degenerative aortopathy.  
• Intervention is guided by family history, if unknown, 4.5-5.0 cm.\textsuperscript{53,60} | 4.5-5.0 cm\textsuperscript{53,60} |

Table 1.2 Etiology-specific details that influence surgical cut-point modifications, and the corresponding intervention diameter recommendations by the Canadian Cardiovascular Society (CCS) and the American College of Cardiology Foundation (ACCF).

1.7 Aortic diameter is not a precise predictor of the risk of aortic complication

Aortic diameter is the main quantitative metric for both the CCS and the ACCF guidelines in the intervention of TAAs. The lack of other metrics is due, in part, to the fact that aortic diameter is the only complication-correlated metric that can be extracted from existing TAA imaging techniques.\textsuperscript{87} Guidelines for the timing of aortic repair are based on clinical observations and historical data of TAD outcomes. Frequently referenced are the previously established hinge point indicating the risk of thoracic aortic complications (Figure 1.8).\textsuperscript{78} However, it is increasingly suggested that aortic diameter is
not a precise nor definite indicator of thoracic aortic dissection or rupture. Multiple assessments of IRAD data have indicated that a significant proportion of aortic dissections may occur before their etiology-associated intervention diameters. Pape et al. found that in Stanford type-A aortic dissections in 60% of cases occurred at aortic diameters <5.5cm and over 40% occurred at diameters <5.0cm. These data are concerning, as the recommended intervention threshold of the most common form of TAA (degenerative), is 5.5cm in diameter (Figure 1.9). In a second study, Kim et al. indicated that perhaps as few as 13% of non-genetically induced dissections occur at an aortic diameter >5.5cm. Furthermore, it should be noted that the dissections estimates performed by Pape et al. and Kim et al. could be conservative. Both studies evaluated patients presenting with acute type A aortic dissections. This focus misses a key demographic of fatal TADs. When individuals with TAD die either before reaching a hospital or before diagnosis is made, aortic diagnostic information is no longer acquired. In Sweden, where autopsies are mandatory for all unexpected deaths, Olsson et al. found that 22% of all TAA and TADs were diagnosed post-mortem upon autopsy. Together, these serve as an indication of the inadequacy of current diameter-based imaging approaches to assess the risk of dissection.
Figure 1.8 Estimated effect of ascending aortic aneurysm size on risk of complication. Reproduced with permission from Coady et al. 1997\textsuperscript{78}
Figure 1.9 Maximal aortic diameters at occurrence of thoracic aortic dissection in Marfan syndrome patients (MFS) and non-Marfan syndrome patients. Red Dashed line indicates common degenerative surgical cut-point of 5.5cm. Adapted from Kim et al. 2014.

1.8 Magnetic Resonance Imaging Theory

1.8.1 Basics of Magnetic Resonance imaging

Magnetic resonance imaging is based on the principle of nuclear magnetic resonance. This process follows three key steps: polarization, excitation, and relaxation of nuclear spin. Certain atomic nuclei, such as the hydrogen nucleus possess a property known as “spin” angular momentum. This property can be imagined as the nucleus spinning around its own axis, although this construct is a mathematical analogy; the
nucleus itself does not spin. These nuclei are capable of generating a magnetic moment and consequently produce a magnetic field with positive and negative poles.\textsuperscript{88}

In the presence of a static magnetic field $B_0$, the magnetic moments of nuclei will become polarized, and tend to align either parallel or anti-parallel to $B_0$. The angular momentum of the nucleus also leads to precession around the $B_0$ axis. There will be a slight excess number of spins aligned parallel to $B_0$, which will create a net magnetization along the direction of $B_0$.

The net magnetization produced by an ensemble of nuclei (e.g. in a tissue) can be manipulated, and used in combination with magnetic gradients to create magnetic resonance images. Excitation occurs through the application of a radio frequency (RF) magnetic field or $B_1$ pulse tuned to the precession frequency of the nuclei (Larmor frequency). The net magnetization vector will rotate around the $B_1$ pulse vector to create magnetization that can be detected and used in the presence of magnetic field gradients to create an image. Once the $B_1$ field is removed, relaxation occurs, and individual nuclei magnetic moments will dephase will return to the equilibrium state either aligned parallel or anti-parallel to $B_0$.\textsuperscript{88,89}

Conventional MRI exploits differing rates of spin relaxation between different tissues to generate contrast. There are two forms of relaxation, longitudinal (or spin-lattice) and transverse (spin-spin) relaxation, respectively described by the time constants $T_1$ and $T_2$.\textsuperscript{90} $T_1$ and $T_2$ relaxation time constants depend on tissue properties as demonstrated by Figure 1.10.
Figure 1.10 Axial T1-weighted (A) and T2-weighted (B) slices of the human brain. Gray matter, white matter, and cerebral spinal fluid have different relaxation time constants leading to contrast in T1-weighted and T2-weighted images. Case reproduced with permission, courtesy of Dr Ahmed Abdrabou, Radiopaedia, rID:22973.

1.9 Diffusion MRI

1.9.1 Diffusion-weighted imaging

Diffusion MRI, while utilizing the same principles of excitation and relaxation, exploits the diffusion-driven displacements of water to generate image contrast.

Brownian motion characterises the random translational motion of a molecule in a fluid or gas as a result of carrying energy.\(^1\) Einstein showed how Brownian motion was linked to the diffusion coefficient of Fick’s laws, thus bridging for the first time the concepts of diffusion and Brownian motion.\(^2\) From a statistical perspective, the displacement (\(\sigma\)) of a particle in a fluid after diffusion time (\(t\)) can be characterized by the diffusion coefficient (\(D\)) of a particle. Due to the random nature of water diffusion, the
typical one-dimensional translational distance away from the point of origin ($\sigma$) is not linear, and can be modeled by the following formula:

\[ < \sigma^2 > = 2Dt \]  

(1)

An important property of free diffusion is that the distribution of particles that start their random walk at the origin is described by a Gaussian function.

At 37°C the self-diffusion coefficient of free water is approximately $3.0 \times 10^{-9}$ m²/s. This diffusion rate translates to about 32% of molecules reaching a distance of 17µm in 50 milliseconds, with only 5% travelling distances greater than 34µm. In biological tissues, however, the actual diffusion of water molecules is reduced to only a few micrometres. Within tissues, water interacts with membranes, fibers, macromolecules, and other cellular/molecular tissue components. Consequently, the distribution of diffusion distance is no longer Gaussian. Diffusion-weighted MRI creates images that are weighted by the amount of diffusion of hydrogen nuclei on water molecules. Different tissue types restrict water diffusion to different extents producing contrast.

The ability of modern MRI to be sensitive to the diffusion of water molecules within tissues is based on the work of Stejskal and Tanner. They introduced a specific diffusion encoding technique by using magnetic field gradients in what is called a pulsed gradient spin-echo (PGSE) sequence. Furthermore, they provided a solution to the Bloch-Torrey equations that included diffusion as a relaxation process. This theoretical
framework demonstrated that the magnitude and phase of the NMR signal is related to diffusivity. This theory, when combined with MRI spatial encoding techniques created the basis for diffusion-weighted MRI.  

The PGSE DWI approach incorporates a $90^\circ - 180^\circ$ spin echo pair of RF pulses with large and equal diffusion-sensitizing gradients placed on either side of the $180^\circ$ pulse. By manipulating the strength of this gradient, we can control the magnitude of diffusion weighting or $b$-factor. DWI is performed by applying these diffusion-sensitizing gradients in each of three orthogonal directions (e.g. along the x-, y-, and z- directions of the MRI scanner).  

This approach produces a series of T2-weighted images that are additionally weighted by loss of signal from the movement of water molecules along the same direction as that of the diffusion gradient. There is no additional signal loss from stationary water molecules. Whereas, in highly mobile water molecules, nuclei accrue random and unique phase changes as they move about within the first diffusion gradient, that cannot be refocussed during the second diffusion gradient, resulting in signal loss in diffusion weighted images.

1.9.2 Diffusion tensor imaging

Diffusion Tensor Imaging (DTI) is an extension of diffusion-weighted imaging and was introduced by Basser and colleagues in 1994. Given that biological tissues are highly complex, the direction and anisotropy of diffusion varies from voxel to voxel. DTI can provide more in-depth information regarding these diffusion parameters. Within this
model, a second order symmetric tensor ($\mathbf{D}$), represented mathematically as a 3x3 matrix corresponding to diffusion rates in each combination of directions characterizes the diffusion in all spatial dimensions for each voxel (Eq. 2). The diffusion tensor describes the diffusion of water molecules using a Gaussian model. By varying the direction of diffusion encoding gradients, anisotropic diffusion can be observed. Diffusion anisotropy can provide insights into tissue microstructure. Diffusion ($D$) represents the diffusion coefficient along each direction and the correlation between these directions. The diffusion tensor can be calculated from DWI data, collected from diffusion-sensitized acquisitions in six or more directions. The use of greater than six gradient directions can improve the accuracy of tangential or off-diagonal information.

$$\mathbf{D} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{bmatrix} \quad \text{(2)}$$

From a given DTI scan, the decreased signal ($S$) (due to diffusion weighting) is compared to the original signal without applied diffusion gradients ($S_0$) in multiple directions to calculate the diffusion tensor:\textsuperscript{96}

$$S_k = S_0 e^{-b g_k D g_k} \quad \text{(3)}$$

Here ($g_k$) is the diffusion gradient direction from 1 to $N$ and ($D$) is the diffusion coefficient in the given gradient direction. The $b$ value influences the extent of diffusion weighting and can be defined as:
\[ b = \gamma^2 G^2 \delta^2 (\Delta - \frac{\delta}{3}) \]  \hspace{1cm} (4)

Here \((\gamma)\) represents the gyromagnetic ratio, \((G)\) represents gradient amplitude, \((\delta)\) represents time of applied gradients and \((\Delta)\) represents time between applied gradients.\(^6\)

The value of a diffusion tensor in this case, is that it can physically be modelled as an ellipsoid, representing the calculated theoretical volume of diffusion within a given voxel.

\[ \begin{align*}
E_1^1 & \hspace{1cm} E_2^2 \\
E_3^1 & \hspace{1cm} E_4^2
\end{align*} \]

\( \lambda_1, \lambda_2, \lambda_3 \)

**Figure 1.11 The diffusion ellipsoid**

Any ellipsoid can be modeled with 4 pieces of information: the primary eigenvector, and 3 eigenvalues representing the orthogonal dimensions of the ellipsoid. These values however are not intrinsic to measurements taken from a DTI scan. We can instead generate \((D)\) (Eq. 2) and manipulate the resultant matrix to calculate these key
parameters. The three orthogonal axes of the ellipsoid can be aligned with the x, y, and z axes of the reference frame. When a diffusion ellipsoid is aligned with the reference frame axes, the tensor describing the ellipsoid is diagonal (Figure 1.11).

\[
\mathbf{D} = \begin{bmatrix}
D_{xx} & D_{xy} & D_{xz} \\
D_{yx} & D_{yy} & D_{yz} \\
D_{zx} & D_{zy} & D_{zz}
\end{bmatrix} = S^{-1} \begin{bmatrix}
\lambda_1 & 0 & 0 \\
0 & \lambda_2 & 0 \\
0 & 0 & \lambda_3
\end{bmatrix} S
\]  

(5)

\[
S = \begin{bmatrix}
\mathbf{E}_1 & \mathbf{E}_2 & \mathbf{E}_3
\end{bmatrix}
\]  

(6)

These diagonal elements now describe the eigenvalues or orthogonal dimensions of the ellipsoid. The diffusion tensor \( \mathbf{D} \) is a real symmetric matrix, and therefore can be diagonalized. That is, there exists a 3D rotation matrix \( S \), Eq. 6 for which the \( \mathbf{D} \) matrix can be transformed such that Equation 5 is satisfied. Here we have gathered all necessary elements to define a diffusion tensor within a voxel: \( \lambda_1, \lambda_2, \lambda_3 \), the eigenvalues and \( \mathbf{E}_1, \mathbf{E}_2, \mathbf{E}_3 \) eigenvectors.

Given the ellipsoid estimation of diffusion, multiple inferences can be made regarding the diffusion characteristics and by microstructure of tissue within a given voxel.

As was previously described, when not inhibited by tissues or other constricting factors, a water molecule’s motion is completely random and the probability for this
molecule to move in any direction is also random. In this case, diffusion is considered isotropic and the probable motion for the water molecule can be represented as a sphere. If however, tissue predisposes diffusion into a particular orientation, this is known as anisotropy. The degree to which tissue causes this deviation in diffusion isotropy can be characterized by DTI using Fractional anisotropy (FA). FA can be estimated from the diffusion tensor and is given by:

\[
FA = \sqrt{\frac{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_1 - \lambda_3)^2}{2(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}}
\]  

(7)

FA ranges from 0 to 1, with 0 for perfect isotropy, such that \(\lambda_1 = \lambda_2 = \lambda_3\), as eigenvalues become progressively more unequal, FA approaches 1 (Figure 1.12).
Figure 1.12 Image depicts the random walk diffusion pattern filling a circular/spherical volume when uninhibited (isotropic diffusion) and the directional diffusion pattern when constrained by adjacent walls/tissues.

It is also valuable to assess the total amount of water diffusion within each voxel. Within the diffusion tensor model this is known as Mean diffusivity (MD) and is given by:

$$MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$$  \hspace{1cm} (8)

FA and MD are the most commonly evaluated diffusion scalars in DTI analyses. However, these metrics are rotationally invariant and independent of eigenvalue sorting. However, given the application of diffusion sensitizing gradients in multiple directions, DTI can provide insights into the directionality of diffusion as well. With regards to the
diffusion ellipsoid. Axial Diffusivity (AD) = \lambda_1 represents the water diffusivity parallel to the primary eigenvector, the main orientation of diffusion. Radial diffusivity (RD) represents the diffusivity orthogonal to the primary axis of diffusion. RD is given by:

\[ RD = \frac{\lambda_2 + \lambda_3}{2} \]  

Furthermore, studying the diffusion orientation can be highly informative. Based upon the primary eigenvector within each voxel, the primary direction of diffusion is commonly colour mapped. This method highlights the favoured direction of diffusion and may provide insights into tissue microstructure and orientations. When the primary eigenvectors of multiple adjacent voxels are connected, in conjunction with high FA, it suggests fibers exist within the tissue. This concept is extended further to visualize fibre tracts using diffusion tractography. Modeled fibers that satisfy a given length and FA requirement can be modeled, such that DTI can estimate and provide information regarding anatomical structures within tissues. This method has been applied to model white matter within the brain.

1.10 Related DTI applications

1.10.1 Cardiac DTI

Although DTI is most commonly applied in studies of the brain, it has previously seen applications in the heart. Myofibers in the subepicardium spiral around the long axis of the heart with a positive helix angle, whereas those in the subepicardium have a
negative helix angle. These orientations are also highly conserved between species. These predominant orientations have been established to be reliably modeled by the primary eigenvector in DTI scans. As such, the angular deviation of primary eigenvector maps from the expected myocyte orientation are known as helix angle maps. Helix angle maps, in addition to DTI scalar maps have been generated for healthy hearts. Anatomically-validated DTI fiber modeling and tractography of the healthy heart has been undertaken. Notably, DTI has also been reported to identify various diseased pathologies of the heart. DTI of the myocardium after ischemic injury has been performed in humans in vivo, with abnormal DTI parameters associated with abnormal myocardium architecture. Furthermore, altered DTI scalar metrics are also associated to myocardial disarray in the case of hypertrophic cardiomyopathy and ventricular arrhythmias.

1.10.2 Arterial DTI

There is a small body of literature exploring DTI and blood vessels. Flamini et al. used DTI to assess fiber orientations in the ex vivo porcine aorta. Tractography fiber angles were found to be altered in frozen porcine aortic tissue in comparison to fresh tissue. Additionally, in an ultra-high field strength cardiovascular DTI study, rat heart and aortic tissue was modeled using tractography at an 100μm isotropic resolution. Here it was observed that abdominal aortic fiber wrapping adopted a largely circumferential orientation. Additionally, during the course of my thesis studies, a porcine carotid artery study assessing component contributions to DTI endpoints was published. Changes in DTI scalar indices were attributed to unquantified histological
modifications. My work aims to extend these cardiovascular DTI assessments by quantitatively correlating DTI scalar indices with aortic medial degeneration and extending aortic DTI endpoints to human TAA samples.

1.11 Purpose and Hypothesis

The purpose of my thesis is to explore the ability of DTI to detect and quantify TAA pathologies. My hypothesis is that TAA aortopathy alters the diffusion properties of ascending aortic tissues and that quantified scalar metrics from DTI can detect these changes. I further hypothesize that DTI scalars metrics, specifically fractional anisotropy and mean diffusivity, will correlate with the extent of medial degeneration in ex vivo samples.

1.12 Thesis overview

The overall objective of this research project was to explore the potential use of MR-DTI to delineate thoracic aortic pathologies in ex vivo samples.

In chapter 2, my main objective was to relate the diffusion characteristics of the porcine ascending aorta with the microstructure of representative porcine ascending aortic tissue. I evaluated the relationship between DTI parameters and aortic tissue damage measured by histology. To model damaged aortic tissue, I developed an enzyme-induced ex vivo porcine model of medial degeneration. Damaged and intact aortas were scanned using an ultra-high magnetic field 9.4T MRI scanner. I performed DTI of ex vivo aneurysmal human ascending aortas obtained from ascending aortic replacement surgery.
DTI-based metrics including fractional anisotropy, mean diffusivity, and radial diffusivity were assessed. These data were correlated with the extent of tissue damage in human aortopathy samples, assessed with quantitative histopathology.

Chapter 3 summarizes the thesis results, outlines the strengths and limitations of DTI in the context of thoracic imaging, and describes possible future work that will aid in the translation of this exciting imaging modality to assess aortic wall structure.
1.13 References


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CHAPTER 2

2 DELINEATION OF ASCENDING AORTIC MEDIAL
DEGENERATION USING DIFFUSION TENSOR IMAGING

2.1 Introduction

Thoracic aortic aneurysms (TAAs) entail a pathologic and progressive dilatation of this component of the aorta.\textsuperscript{1,2} If not addressed, TAAs can lead to dissection or rupture, both potentially fatal complications if not promptly treated.\textsuperscript{3,4} To prevent these potentially fatal complications, those who are deemed at risk will undergo prophylactic surgery to replace the dilated segment of aorta with a graft.\textsuperscript{5,6} The primary factor utilized in this risk assessment is aortic lumen diameter as determined by non-invasive thoracic aortic imaging. Computed tomography, echocardiography or MRI all are valid imaging modalities used to assess the progression of TAAs.\textsuperscript{7,8} These imaging modalities allow for the assessment of aortic diameter and overall vessel morphology. Most important is aortic diameter, which is monitored to identify if it has reached a threshold for surgical intervention.\textsuperscript{5,6,9}

However, evidence has shown that aortic diameter is not a precise predictor of adverse TAA complications.\textsuperscript{10} Data from the International Registry of acute Aortic Dissections (IRAD) have revealed that the median aortic diameter for patients presenting with ascending aortic dissection was below the commonly recommended aortic intervention diameter of 5.5 cm.\textsuperscript{10,11} Thus, based on existing assessment and intervention
criteria, many aortic complications may occur prior to surgical treatment. Lowering the existing intervention threshold would capture an increased portion of aortic complications. However, lowering surgical cut-points would also subject a large number of patients in the general population to unnecessary aortic replacement, exposing them to the surgical risk without offering any benefit.

A key underlying phenomenon leading to TAA formation and consequent dissections is aortic medial degeneration. Compositional changes in the aortic media can progress and lead to structural weakness. Hallmark TAA pathology includes smooth muscle cell (SMC) loss, SMC disarray, elastin breakdown, and the accumulation of glycosaminoglycans (GAGs). Although existing TAA imaging modalities provide information regarding aortic morphology and dimensions, none provide direct insight into aortic wall components. This is a major shortcoming of current TAA imaging modalities.

An imaging modality that provides data on aortic medial microstructure could, in theory, improve TAA risk assessment. Diffusion tensor imaging (DTI) is an MRI imaging modality that tracks multi-directional water movement within tissues. Due to the restriction in water diffusion characteristics within tissues, in contrast to unrestricted water diffusion, DTI can delineate cellular structure and microstructure within certain tissues. The organized cellular arrangement of the normal thoracic aorta raises the possibility that DTI may reveal architectural details of the aortic wall. Recent diffusion studies of porcine arterial structure support this possibility.

I hypothesized that DTI scalar metrics can identify aortic medial degeneration, the underlying pathology of TAA formation. Healthy porcine aortic tissue was first assessed
to interrogate aortic medial tissue diffusion characteristics. Subsequently, an *ex vivo* porcine model of medial degeneration was developed. Images of these samples were obtained using DTI and segmented scans were compared to corresponding histologic sections. Finally, a cohort of human thoracic aortic samples was harvested from patients with TAAs and images were also obtained with DTI.

I report that using *ex vivo* imaging at a magnetic field strength of 9.4 T, aortic wall directional architecture can be detected by DTI. Diffusion tensor scalar indices detected medial degeneration in both porcine and human ascending aortas. These findings raise prospects for non-invasive imaging of the aortic wall beyond aortic dimension.

### 2.2 Methods

#### 2.2.1 Porcine aortic sample preparation

The porcine aortas used in this study were harvested from tissue provided by Mount Brydges Abattoir, London, Ontario, Canada. Aortas were harvested from six-month-old Yorkshire pigs. Aortas were removed from pigs with hearts attached. Samples were transported and stored in 1x phosphate buffered saline (PBS) at 4 ℃. Aortic samples were separated from the heart above the aortic sinus. Excess fatty tissue, and lymph nodes were removed. Ascending aorta, i.e. the portion between the sinotubular junction and the aortic arch, was divided into 2~3 segments; each segment was about 1.5 cm long.
2.2.2 Porcine Aorta Model of Medial Degeneration

An enzyme-mediated degeneration approach was undertaken to induce degenerative damage of porcine aortic media. Samples were injected with a collagenase-elastase cocktail to create a localized intra-medial degeneration zone. Injections were performed using a 25G needle via the adventitia, with an approximate 45° angle between the needle and the outer aortic surface and terminating approximately halfway into the aortic wall thickness. Each injection was 30 μL in volume and contained 0.1% collagenase type-II (Worthington Biochemical Corporation, Lakewood, NJ, USA) by mass and 12.5 active units of porcine pancreatic elastase (Millipore Sigma, Oakville, Ontario, Canada). Samples were incubated at 37 °C for 30 minutes and subsequently washed twice with 1x PBS to remove excess enzyme. Samples were incubated in M199 cell culture medium (ThermoFisher Scientific, Waltham, MA, USA) with 10% FBS at 37°C for 15 hours. Samples were washed again with 1x PBS prior to MR imaging.

2.2.3 Human Aortic Samples

Aortic tissue was harvested from individuals undergoing ascending aortic replacement, or cardiac transplantation in accordance with protocols approved by the Institutional Review Board/ Research Ethics Committee at Western University. Written informed consent was obtained from all patients with the exception of one heart transplant recipient. In this case, normally discarded tissue was harvested and there were no patient identifiers obtained. A summary of the patient samples is presented in Table 1. Maximal ascending aortic diameter was recorded from the double-oblique short axis
plane of contrast enhanced multidetector CT images. Aortic samples were fixed with 10% neutral-buffered formalin within 30 min after resection. After 48 hours of fixation, samples were subsequently scanned by MRI.

2.2.4 MRI Hardware and Scanning Protocol

Images were acquired using a 9.4 T Bruker small animal MRI scanner at the Centre for Functional and Metabolic Mapping located within the Robarts Research Institute at the University of Western Ontario. Anatomical images were acquired for each specimen at the beginning of each session using a T2-weighted TurboRARE2D pulse sequence (32 averages, 35 slices, slice thickness = 500 μm, FOV 32x32 mm, matrix size 160x160, in-plane resolution = 200x200 μm, TE = 20 ms, TR = 7 s, echo spacing = 10 ms, rare factor = 8). The DTI scans were acquired using a 2-dimensional, multi-shot, spin echo, echo-planar-imaging (SE-EPI2D) pulse sequence (4 shots, 35 slices, slice thickness = 500 μm, FOV 32x32 mm, matrix size 160x160, in-plane resolution = 200x200 μm, TE = 25 ms, TR = 7 s). Total 60 directions with b-value = 1000 s/mm² were obtained using the following diffusion gradient parameters: gradient strength (G) = 250 mT/m, time between the start of the first and second diffusion pulse (Δ) = 13.5 ms, the duration of a single gradient pulse (δ) = 4.5 ms. Five b = 0 s/mm² scans were evenly distributed throughout the acquisition. Acquisition duration was 1 hour and 12 minutes per average. Seven averages were used to improve signal-to-noise ratio. Porcine specimens were scanned in 50 mL centrifuge tubes, while larger human aortic specimens were scanned in 60 mL, 50 mm diameter wide-mouth polycarbonate jars (Fisher scientific, Waltham, MA, USA). In the case of human aortic samples, an iodine marker
was placed outside of the ring to indicate the position of a surgical suture. Porcine aortic samples were scanned using a 30 mm RF volume coil and larger human samples were scanned using a 50 mm RF volume coil. Specimens were secured in their respective containers with gauze and subsequently submerged with low viscosity, inert fully fluorinated oil (Christo-lube MGC 1009, Engineered Custom Lubricant, Aurora, IL, USA).

3 T DTI scans were acquired using a 3 T Siemens MAGNETOM Prisma Fit whole-body MRI scanner at the Centre for Functional and Metabolic Mapping located within the Robarts Research Institute at the University of Western Ontario. The DTI scans were acquired using a 2-dimensional, multi-shot, echo-planar imaging (RESOLVE) pulse sequence (single shot, slice thickness = 8 mm, FOV 161.6×161.6 mm, matrix size 202×202, in-plane resolution = 800 × 800 μm, TE = 59 ms, TR = 150 s). Total 12 directions with b-value = 350 s/mm² were obtained. Eight b = 0 s/mm² scans were evenly throughout the acquisition. Thirty-two averages were used to improve signal-to-noise ratio.

2.2.5 DTI analysis

Images were pre-processed using fMRI Software Library (FSL, v5.0.10, Oxford, UK). The EDDY utility was used to correct for eddy current-induced distortions as well as susceptibility-induced distortions. Subsequently the DTIFIT toolkit was used to generate the following scalar maps: fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), primary eigenvector direction (Colour and line interpretations).
Tractography fiber modeling was performed using MRTRIX3\textsuperscript{16} using a deterministic tractography algorithm with an FA cut-off minimum of 0.1 and a maximum angle $\theta$ between successive steps of 30°. Here, seeds, or tract origins, were placed using a rejection sampling algorithm. This allowed the placement of tracts, in areas that are most expected to contain anatomical fibers, based on the minimum streamline rejection criteria for this fiber model. A maximum of 5000 seeds were permitted per tractography model.

Tensor angle maps were generated using an in-house developed MATLAB (MathWorks, Natick, MA, USA) code. Briefly, primary eigenvector maps, as output from DTIFIT, were imported into MATLAB. Then, the inner voxels of the aortic ring were identified and marked to indicate the intimal boundary. Subsequently a cubic spline interpolation function was fit to every 5\textsuperscript{th} voxel along the intimal boundary to provide an approximation of the inner morphology of the aortic ring. Here, tangent lines could be approximated at any point along the spline function. Subsequently, the shortest distance between each tensor/voxel and the spline was assessed. Here the difference between the spline tangent angle and the 2D component of voxel 1\textsuperscript{o} eigenvector was calculated and mapped as a “2D tensor angle”.

2.2.6 Histological staining and Analysis

Immediately after scanning, aortic specimens were placed in formalin for another 48 hours at minimum. To ensure that full-ring histology slides could be compared to the slice of equivalent depth from MRI scans, the aortic sample was histologically processed and embedded in the correct orientation such that the initial full-ring histological sections
corresponded to the first full-ring MRI slice within the acquired stack. Two serial sections from each sample were stained with Hematoxylin and Eosin (H&E), and Movat’s pentachrome stain. Whole-slide brightfield imaging was subsequently performed on all stained sections using a Leica Aperio AT2 slide scanner (Lincolnshire, IL, USA) in the Molecular Pathology Core Facility, Robarts Research Institute, Western University.

Regions of interest (ROIs) were assigned to stained histological sections using QuPath bioimage analysis software (v0.2.3). To ensure ROIs occurred in identical locations between H&E and Movat’s pentachrome slides, the identical QuPath annotation file was used to assess ROIs for both H&E and Movat’s Pentachrome-stained sections. ROIs were then exported for analysis via Fiji biological-image analysis software (v1.53c). Smooth muscle cell density analyses were performed via region and sample-blinded cell nuclei counting on H&E-stained tissue sections using the Fiji Cell Counter plug-in. Elastin ROI quantification was performed using the Fiji Color Threshold plug-in to assess Movat’s pentachrome-stained ROIs. Here the hue-saturation-brightness profiles were thresholded in order to only identify elastin-containing-pixels (Hue 130:255, Saturation 132:255, Brightness 0:53), and GAG-containing pixels (Hue 96:185, Saturation 21:176, Brightness 100:255). Subsequently, the area fractions of elastin and GAG were calculated as percent of pixels within a given ROI.

2.2.7 Histology ROI assignment

For each unique DTI-histology assessment, H&E-stained sections of porcine enzyme-mediated degenerated samples (n=3) were annotated with 80 ROIs. Each ROI
was 62500 \( \mu \text{m}^2 \). An array of ROIs was assessed within, and surrounding the injection zone, with a minimum of 250 \( \mu \text{m} \) between ROIs in any direction. The identical ROI annotation grid was then applied to the digital histology from the successive histological section stained with Movat’s pentachrome.

To compare all human aortic samples (n=8), each sample, was ten ROIs were assigned about the aortic wall. ROIs were centered on the aortic media, 4 mm\(^2\) in area, and distributed equidistantly about the circumference of each aortic ring on both H&E and Movat’s pentachrome-stained digital histology images.

### 2.2.8 Histology-MRI correlation

ROIs were assigned to brightfield histology as described above, and histological traits of each ROI were analyzed. Alignment and segmentation of corresponding DTI scalar maps were performed in 3D Slicer (v4.11.0). The whole-slice brightfield microscopy image, including ROI annotation, was imported into 3D Slicer. Subsequently, the whole-ring slice, corresponding to the depth of the annotated histology section was also imported. The two images were co-registered using a linear, non-rigid algorithm from the 3D Slicer “Landmark Registration” module. Landmarks were assigned based on the position of a surgical suture and aortic ring morphology.

Once aligned, the “Segment Editor” module was used to assign quantifiable ROIs on MRI maps to corresponding ROIs on histology. The “Segment Statistics” module enabled quantification of the voxel-averaged mean for a given segment/ROI. For porcine ROI comparisons, each histological ROI was compared to a single voxel from a region-
matched segmentation. For human ROI comparisons, each histological ROI was compared to a 48-voxel, region-matched ROI. Correlations and regressions between histological ROIs and corresponding segmented regions on DTI maps were performed using GraphPad Prism (v.8.4) (La Jolla, California, USA).

2.3 Results

2.3.1 Identification of diffusion in porcine ascending aortas using diffusion tensor imaging

I first sought to establish the diffusion tensor characteristics of normal ascending aortic tissue. 2-3cm segments of the porcine ascending aortic tissue from 6-month-old Yorkshire pigs were harvested. The isolated tissue samples (n = 4 aortas) were scanned with MRI at a field strength of 9.4 T. Figure 2.1 depicts a full-ring 500 µm slice from a scan of a healthy porcine aorta. Despite the risk of low signal to noise and potential artifacts inherent to scanning hollow tissues, T2-weighted and notably DTI scans demonstrated a high quality, artifact-free image (Figure 2.1A, Figure 2.1B).

MD and RD values were consistent within the media. Aortic adventitia, the outermost part of the aorta, lacks layered structure. Accordingly, voxels representing adventitial tissue had higher mean and radial diffusivity on the outer edge of the ring (Figure 2.1C, Figure 2.1D). FA maps demonstrate that porcine aortic media is an anisotropic tissue (Figure 2.1 E). In order to understand the distribution of DTI scalar metric magnitudes from aortic sample medial voxels, the media-containing voxels from healthy porcine samples were graphed using a violin plot (Figure 2.2). Plots show the
median of MD, RD and FA scalar metrics 0.00065 ± 0.00024 mm²/s, 0.00056 ± 0.00027 mm²/s, and 0.43± 0.15 respectively) as well as the distribution of all medial voxels for the 4 healthy porcine aortic samples. Complete descriptive statistics regarding control porcine tissue provided in Table 2.1. The similarity of DTI scalar metrics among the 4 different porcine samples indicates that aortic tissue is a promising tissue model of the human aorta in which variations in diffusion characteristics within the aortic wall might be detectable.

<table>
<thead>
<tr>
<th></th>
<th>Pig 1</th>
<th>Pig 2</th>
<th>Pig 3</th>
<th>Pig 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Voxel count</strong></td>
<td>1741</td>
<td>3250</td>
<td>4834</td>
<td>4079</td>
</tr>
<tr>
<td><strong>Mean MD</strong></td>
<td>0.00069± ±0.00031</td>
<td>0.00062± ±0.00019</td>
<td>0.00064± ±0.00024</td>
<td>0.00074± ±0.00026</td>
</tr>
<tr>
<td><strong>Mean RD</strong></td>
<td>0.00056± ±0.00031</td>
<td>0.00046± ±0.00016</td>
<td>0.00053± ±0.00024</td>
<td>0.00067± ±0.00031</td>
</tr>
<tr>
<td><strong>Mean FA</strong></td>
<td>0.39± ±0.15</td>
<td>0.47± ±0.14</td>
<td>0.35± ±0.13</td>
<td>0.50± ±0.15</td>
</tr>
</tbody>
</table>

Table 2.1 Mean DTI scalar values from the media of each of 4 control porcine ascending aortas.
Figure 2.1 Full-ring slices of MRI scans and diffusion tensor imaging (DTI) scalar maps from porcine ascending aortic tissue. A. T2-weighted MRI scan B. DTI scan of the same ring C. Mean diffusivity map D. Radial diffusivity map E. Fractional anisotropy map. Scans were acquired at a spatial resolution of 200 μm$^2$ and a slice thickness of 500 μm.
Figure 2.2 DTI voxel scalar magnitudes in porcine aortas. Violin plots depict the magnitude distribution of mean diffusivity (MD, left), radial diffusivity (RD, middle), and fractional anisotropy (FA, right). Each plot represents DTI scalar magnitudes from the medial region of slices from 4 different samples, total voxels = 13,808. Wide dashes indicate median and narrow dashes denote the interquartile range.
2.3.2 Diffusion tensor orientation in the porcine aorta is circumferential

Given the layered and directional structure of the aortic wall, I next sought to determine if the diffusion tensor orientations in porcine aortas reflects the histological structure. Primary diffusion eigenvector maps derived from DTI scans (Figure 2.3) demonstrated that a net orientation of diffusion within voxels could be ascertained. Colour mapping of primary eigenvector orientations depicts the arrangement of diffusion tensors around the circumference of the aortic ring (Figure 2.3A). Furthermore, line representations of primary eigenvectors show excellent alignment between adjacent voxels (Figure 2.3B). Overall, this circumferential alignment mimics the layered and circumferential organization of lamellar units in identical locations of the aortic ring as shown in Figure 2.3 C&D.

As noted, visually we found that the orientation of primary eigenvectors wraps circumferentially, in line with aortic morphology. To verify this phenomenon, we developed a quantitative approach to measure the alignment between the orientation of tensors and orientation of the aortic wall. Two-dimensional (2D) tensor angles were measured to quantify the deviation of diffusion tensor orientations from the direction of the circumferential axis of the aortic ring, which was measured as the tangent of the intimal surface on MRI scans. Evaluation of 2D tensor angles in control porcine tissue indicated that tensors do conform tightly to the aortic wall orientation (Figure 2.4). Figure 2.4A depicts the mapping of quantified 2D tensor angles within a healthy porcine ascending aortic sample. The tensor angles correlated tightly with the circumferential axis.
Figure 2.3 Diffusion directions in harvested porcine aorta. A. Primary diffusion orientation map of a full-ring slice from a porcine aorta. Colours are indicative of primary eigenvector direction. B. Zoomed image of the framed region from (A). Lines correspond to tensor orientation within a given voxel. C-D. Region-matched area from control porcine aorta stained with hematoxylin and eosin (C) and Movat’s pentachrome stain (D). Elastin and smooth muscle cell orientations are similar to quantified tensor orientations.
Figure 2.4 2D tensor angles derived from diffusion tensor imaging scan of the porcine aortic ring. A. Map of 2D angles between tensor orientation and the tangent of the inner perimeter corresponding to the voxel location from a representative slice of a control porcine ascending aorta. B. Histogram of tensor angles from the slices in 4 control porcine ascending aortas, total voxels = 13,808. Tensor angles appear are distributed normally about 0° indicating that tensor orientations align with the approximate morphology of the aortic ring. Black line depicts a Gaussian-least squares regression of frequency distribution (R²=0.9945).
of the sample. I further examined the distribution of voxel 2D tensor angles from healthy porcine tissue samples (n=4, Figure 2.4B). The frequency histogram indicates a tight, normal distribution of tensor angles about 0°. A Gaussian, least squares fit of the frequency histogram indicated a mean=0.32° and a standard deviation=8.297°. These findings indicate 2D tensor angles conform to the aortic morphology in healthy aortic samples, a phenomenon which could potentially be disrupted in the case of disorganized or diseased aortic tissues.

I next investigated the utility of diffusion tensors to model the 3D organization of the aorta. DTI tractography was generated using MRTRIX3, as a fiber model of diffusion within the aortic wall (Figure 2.5). Consistent with the primary eigenvector maps (Figure 2.3B), fiber tractography depicts a circumferential arrangement of modeled fibers. An in-plane view of 3D fiber tractography from a healthy porcine aorta shows circumferential wrapping of tracts (Figure 2.5A). Figure 2.5B shows fiber tractography with 30° Y-axial rotation from Figure 2.5A. Figure 2.5C shows fiber tractography from the same model as shown in Figure 2.5A, rotated by 90°. Here it can be noted that the wrapping of fiber tracts is not purely circumferential, instead following a slight pitch angle from the vertical axis. Images of tractography provide a strong sense of the continuous nature of modeled fibers.
Figure 2.5 3D Fiber tractography of porcine ascending aortic sample. A. In-plane orientation of fiber tracts. B. Tracts rotated 30° about the Y-axis in the x-z plane. C. Tracts rotated 90° about the Y-axis in the x-z plane. Tractography indicates fibers adopt a circumferential orientation with a slight longitudinal pitch.
spanning an extensive proportion of the aortic circumference. For the particular aortic sample shown, average tract length was $27.2 \pm 7.6$ mm, indicating the average tract may traverse up to 51% of the aortic circumference before terminating. Average tract length for all untreated porcine samples ($n=4$) was $24.4 \pm 6.5$ mm.

### 2.3.3 Medial degeneration pathology in porcine aorta can be generated by a localized enzyme injection

Having established that DTI mapping provides quantitative data on for the ordered architecture of healthy porcine ascending aorta. I next evaluated whether DTI could detect pathological changes in the ascending aortic media. To mimic the degenerative pathology in human ascending aortic aneurysm\textsuperscript{14}, I developed a porcine model of aortic medial degeneration, induced by localized enzyme injection. Porcine ascending aortas were injected with an enzyme cocktail of elastase and collagenase II. This produced a gradient of medial degeneration histopathologies. Figure 2.6A shows a whole slide image of a Movat’s pentachrome-stained porcine aorta subjected to enzyme-mediated medial degeneration. The medial degeneration was severe at the injection site, and less severe as the circumferential distance from the site of injection increased. Distant locations from the injection site (Figure 2.6B) displayed entirely normal aortic histology. Thick elastin lamellae remained arrange in a parallel, organized fashion and between elastin lamellae are bipolar, elongated smooth muscle cells. The “unaffected distal” zone began about 6 mm from the mid-injection site. Adjacent to this was a “transition zone” (Figure 2.6C) with increased elastin lamellar spacing, mild elastin thinning and some
breaks, and abnormal cell morphologies, such as atrophic muscular cell body, or loss of bipolar elongation. The “border zone” as depicted in Figure 2.6D was also adjacent to the mid-injection zone. Here, there was substantial elastin thinning, fragmentation and loss. Smooth muscle cells had abnormal cell morphologies, with loss of typical layered alignment. Finally, the “mid-injection zone” (Figure 2.6E) exhibited a complete dropout of all elastin and smooth muscle cells, leaving only disorganized extracellular matrix at the center of the lesion. This gradient of aortic degenerative pathology was successfully reproduced in 20 aortic segments each with their own injection.

2.3.4 Medial degeneration can be detected and quantified via DTI

With this established gradient of porcine aortic degeneration, I next sought to determine the ability of DTI to detect these lesions. Enzyme-treated porcine ascending aortas were subjected to DTI at a field strength of 9.4 T. Following the scanning, the whole aortic rings were fixed in formalin, processed, embedded, sectioned and stained with Movat’s pentachrome. This procedure permitted comprehensive and accurate spatial comparisons between whole-aorta histology and DTI scalar maps. Figure 2.7A depicts a Movat’s pentachrome-stained enzyme-injection aortic sample (distinct from Figure 2.6). Figure 2.7B depicts a zoomed-in region, and QuPath was employed to demarcate the site of injection and surrounding regions with an array of ROIs. I found that DTI scalars detected enzyme-induced changes in medial
Figure 2.6 Aortic degeneration pathologies induced by intra-medial enzyme injection. A. Movat’s pentachrome-stained histology of enzyme-injected porcine aorta. B. Unaffected zone depicting elastin lamellar organization and abundant, bipolar, elongated smooth muscle cells. C. Transition zone depicting increased elastin lamellar spacing, elastin breaks, and abnormal cell morphologies. D. Border injection zone depicting severe fragmentation and loss of elastin, with disarray of smooth muscle cells. E. Mid-injection zone depicting complete degradation of elastin and extensive cell loss.
tissue composition. Specifically, the reproducible patterns of the three DTI scalars, MD, RD and FA, for intact control aorta (see Figure 2.1), were disrupted at the site of injection (Figure 2.7 C-E). The zone of altered DTI scalar indices corresponded to histologically-detected aortic degeneration zone. MD and RD scalar maps (Figures 2.7 C, D) displayed a notable *increase* in diffusivity at the site of the lesion. The FA scalar map depicted a notable *reduction* in FA at the site of the lesion (Figure 2.7E). These directional changes in DTI scalars are consistent with histological evidence of damaged aortic microarchitectures.

To further quantify the relationship between histological features and DTI scalar data, I first developed a systematic approach to obtain the quantitative information of histological features. Elastin content, and smooth muscle cell density were quantified from the 3 enzyme-injected porcine ascending aortas. A matrix of ROIs was applied to cover sections from the injection site and adjacent medial tissue for each aorta. The histological features of each ROI were quantified, and subsequently correlated to DTI scalar data via co-registration with the scalar maps and segmentation using 3DSlicer.

Elastin was quantified by colour thresholding ROIs from Movat’s pentachrome-stained sections and expressed as percent area of elastin signals for each ROI (Figure 2.8). Mean diffusivity and radial diffusivity demonstrated strong inverse correlations with the elastin content within a given region of aortic samples (p<0.0001, $R^2=0.53$, $\beta=-1.36 \times 10^{-5}$; p<0.0001, $R^2=0.59$, $\beta=-1.58 \times 10^{-5}$). Conversely, FA displayed a strong positive correlation to elastin content (p<0.0001, $R^2=0.48$, $\beta=6.57 \times 10^{-3}$).
Figure 2.7 Histology and corresponding DTI scalar maps of an enzyme-injected porcine ascending aorta. A. Whole slide image depicting a Movat’s pentachrome-stained section of an enzyme-injected porcine aorta. B. Zoomed region corresponding to red box in (A) depicting histological region of interest layout for MRI-histology correlations. C-E. Maps of mean diffusivity (C), radial diffusivity (D), fractional anisotropy (E) corresponding to a representative slice from porcine aorta with enzyme injection. Deviations in diffusivity and anisotropy in scalar maps correspond with the site of enzyme-mediated degeneration.
Figure 2.8 Relationships between DTI scalar metrics and elastin content in enzyme-damaged porcine aortas. A-C. Scatter plot and linear regression lines depicting the correlation between mean diffusivity (A), radial diffusivity (B), fractional anisotropy (C) and histologically quantified elastin content. Each point represents values from a region of interest derived from co-registered MRI scan and histology. Points of different colours indicate values derived from different samples. Data were obtained from 3 different samples. Each sample was quantified using 100 regions of interest.
I further assessed the relationship between DTI scalars and smooth muscle cell density, the latter using H&E-stained sections (Figure 2.9). MD regression showed a strong inverse correlation to smooth muscle cell density (p<0.0001, R²=0.45, β=-0.10). Similarly, RD demonstrated a strong inverse correlation to smooth muscle cell density (p<0.0001, R²=0.45, β=-0.19). Regression of smooth muscle cell density on FA revealed a strong positive correlation (p<0.0001, R²=0.29, β=112.4).

I also assessed the relationship between the orientation of diffusion tensors and aortic wall histopathology. Figure 2.10 depicts the primary eigenvector map from a representative sample subjected to enzyme-induced medial degeneration. Tensor orientations deviated notably from the expected circumferential pattern. 2D tensor angle maps also indicated this change, with tensor angles around the site of injection profoundly deviating from the approximately 0° green signal in non-injected tissue (Figure 2.11 A,B). This is further illustrated by the frequency histogram shown in Figure 2.11C, (mean=0.52°, amplitude=12.30%, standard deviation=13.73°, R²=0.93). There is an increase in voxels displaying large absolute tensor angles of large magnitudes (e.g. 40°-80°).
Figure 2.9 Relationships between DTI scalar metrics and smooth muscle cell density in enzyme-damaged porcine aortas. A-C. Scatter plot and linear regression depicting the correlation between mean diffusivity (A), radial diffusivity (B), fractional anisotropy (C) and histologically quantified smooth muscle cell density. Each point represents a value from a region of interest derived from co-registered MRI scan and histology. Points of different colours indicate values derived from different samples. Data were obtained from 3 different samples. Each sample was quantified using 100 regions of interest.
Figure 2.10 Primary diffusion orientations are disrupted in regions of enzyme-induced medial degeneration. A. Primary diffusion orientation map of a slice from enzyme-injected porcine aorta. Colours are indicative of primary eigenvector direction. B. Zoomed-in view of framed region from (A), lines correspond to tensor orientation within a given voxel.
Figure 2.11 2D tensor angles in degraded porcine aorta. A. 2D tensor angle map of a representative slice from an enzyme-degenerated porcine aortic sample. Large deviations in tensor angle can be noted throughout the injection site. B. Movat’s pentachrome-stained histology of sample corresponding to the tensor angle map shown in (A). C. Histogram of 2D tensor angles from representative single slices of a total of 3 enzyme-treated porcine aortic samples, with a total of 9371 voxels.
2.3.5 The ascending aortic media of patients with thoracic aortic aneurysms display diverse pathologies

Given the correlations between DTI readouts and aortic medial degeneration in the porcine aorta model, I next sought to determine if human TAA histopathologies can be characterized and quantified using DTI. For this study, I obtained 8 human ascending aortic samples retrieved from patients undergoing cardiac surgeries. The samples included 6 aneurysmal aortas from patients receiving ascending aortic replacement procedure and 2 non-aneurysmal aortas from heart transplant recipients. The patient demographics of scanned samples are provided in Table 2.2.
<table>
<thead>
<tr>
<th>Sample I.D.</th>
<th>Aortic Lumen Diameter</th>
<th>Sex</th>
<th>Age</th>
<th>Etiology</th>
</tr>
</thead>
</table>
| 360        | 50                    | M   | 66  | • Bicuspid aortic valve  
|            |                       |     |     | • Aortic stenosis  
|            |                       |     |     | • Ascending aortic aneurysm |
| 361        | 64                    | M   | 79  | • Mega aortic syndrome  
|            |                       |     |     | • Chronic aortic dissection |
| 319        | 24                    | M   | 20  | • Heart transplant recipient |
| 371        | N/A                   | N/A | N/A | • Heart transplant recipient |
| 375        | 52                    | M   | 80  | • Degenerative  
|            |                       |     |     | • Ascending aortic aneurysm |
| 377        | 55                    | M   | 59  | • Degenerative  
|            |                       |     |     | • Severe aortic stenosis  
|            |                       |     |     | • Ascending aortic aneurysm |
| 380        | 56                    | M   | 71  | • Degenerative  
|            |                       |     |     | • Aortic valve replacement |
| 370        | 54                    | M   | 55  | • Bicuspid aortic valve  
|            |                       |     |     | • Ascending aortic aneurysm |

Table 2.2 Patient characteristics.
Aneurysmal human aortic samples displayed a range of histopathological characteristics of medial degeneration, as assessed by H&E, and Movat’s pentachrome staining. There was heterogeneity in aortic medial findings among aneurysmal aortas from different individuals (Figure 2.12). For instance, the aorta of patient 380 contained regions of relatively healthy medial tissue, with elongated and aligned smooth muscle cell (Figure 2.12A), whereas H&E staining of the aorta from patient 360 revealed regions with widespread smooth muscle cell disarray (Figure 2.12B). Figure 2.12C shows that the aorta of patient 377 displayed mild disruption of the elastin lamellar structures, strong smooth muscle cell staining, and limited deposition of glycosaminoglycan. Figure 2.12D shows that the aorta of patient 360 displayed extensive elastin thinning and breaks associated with interlamellar glycosaminoglycan deposition with the presence of smooth muscle cells. Figure 2.12E depicts the aorta of patient 375, which displayed diffuse medial glycosaminoglycan deposition and a large pool of subintimal glycosaminoglycan. There was also elastin disarray and smooth muscle cell drop-out. The aorta of patient 319 also showed inter-lamellar glycosaminoglycan deposition, elastin disruption and smooth muscle cell loss (Figure 2.12F).

2.3.6 DTI scalar metrics detect differences in aortic medial degeneration

The magnitude of slice-averaged DTI scalar metrics of healthy porcine tissue was similar from sample to sample. Within our human cohort, we noted a much larger disparity in average slice DTI scalar magnitudes (Table 2.3). To begin the DTI assessment of human samples, I first evaluated DTI scalar maps from samples whose
Figure 2.12 Histopathology of human ascending aortas. A. Hematoxylin and eosin (H&E)-stained section depicting elongated smooth muscle cell (SMC) nuclei and layered aortic medial organization. B. H&E-stained section depicting irregular SMC nuclei morphologies and orientations. C. Movat’s pentachrome-stained section displaying SMC staining and thick elastin layers with no medial degeneration. D. Movat’s pentachrome-stained section with evidence of elastin lamellae thinning, and inter-lamellar deposition of GAGs. E. Movat’s pentachrome-stained section depicting substantial intimal deposition of GAGs. F. Movat’s pentachrome-stained section depicting elastin disorganization and interlamellar GAG deposition.
histology revealed contrasting levels of disease in the aortic wall. Movat’s pentachrome histology from sample 370 showed little evidence of medial degeneration with clear lamellar organization, limited elastin breaks, strong smooth muscle cell staining and limited evidence of glycosaminoglycan deposition (Figure 2.13A). Conversely, histology from sample 361 revealed evidence of extensive medial degeneration (Figure 2.13E) with extensive elastin breaks, with diffuse proteoglycan deposition throughout the entire media and a reduction in smooth muscle cell density. These stark differences in tissue composition appeared to be detected by DTI scalar metrics. MD maps revealed a lower magnitude of mean diffusivity in the aortic wall of sample 370 compared to sample 361 (mean MD = 0.00072 ± 0.00006 mm²/s and 0.0010 mm²/s ± 0.0001 respectively) (Figure 2.13 B&E). FA maps of these two samples revealed the opposite trend. Sample 370 displaying higher FA than the more damaged sample 361 (mean FA = 0.41 ± 0.07 and 0.35 ± 0.05 respectively) (Figure 2.13 C&F).

2.3.7 Relationship between DTI scalar indices and the elastin content of human aortic media

I next sought to extend and quantify these findings for all samples within our human aortic cohort and evaluate the quantitative relationship between DTI scalars and the extents of human aortic medial pathologies. I demarcated 10 equidistant ROIs around the whole circumference of each human aortic ring stained. The elastin content within each ROI was quantified and compared to voxel-averaged DTI scalars via co-registration and segmentation within 3DSlicer. Region-quantified elastin was plotted against voxel-
<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Mean MD ± SD (mm²/s)</th>
<th>RD ± SD (mm²/s)</th>
<th>FA ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>319</td>
<td>0.00083 ± 0.000085</td>
<td>0.00063 ± 0.000072</td>
<td>0.48 ± 0.072</td>
</tr>
<tr>
<td>360</td>
<td>0.00078 ± 0.00017</td>
<td>0.00060 ± 0.00015</td>
<td>0.45 ± 0.073</td>
</tr>
<tr>
<td>361</td>
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<td>0.00082 ± 0.00010</td>
<td>0.38 ± 0.074</td>
</tr>
<tr>
<td>370</td>
<td>0.00073 ± 0.000055</td>
<td>0.00057 ± 0.000048</td>
<td>0.41 ± 0.065</td>
</tr>
<tr>
<td>371</td>
<td>0.00084 ± 0.000134</td>
<td>0.00063 ± 0.000064</td>
<td>0.45 ± 0.076</td>
</tr>
<tr>
<td>375</td>
<td>0.00080 ± 0.000090</td>
<td>0.00064 ± 0.000095</td>
<td>0.38 ± 0.06</td>
</tr>
<tr>
<td>377</td>
<td>0.00069 ± 0.00013</td>
<td>0.00054 ± 0.00011</td>
<td>0.40 ± 0.07</td>
</tr>
<tr>
<td>380</td>
<td>0.00067 ± 0.00013</td>
<td>0.00051 ± 0.00012</td>
<td>0.43 ± 0.066</td>
</tr>
</tbody>
</table>

Table 2.3 Mean DTI scalar metric values from the media of each of the human ascending aorta samples. Values represent all the voxels from the representative MRI slice selected for comparison with histology.
Figure 2.13 Ascending aorta histology and corresponding DTI scalar maps. **A.** Movat’s pentachrome-stained histology from human ascending aorta showing little evidence of medial degeneration, with SMCs and thick elastin fibers. **B.** Mean diffusivity map from DTI scan corresponding to (A). **C.** Fractional anisotropy map from DTI scan corresponding to (A). **D.** Movat’s pentachrome-stained histology showing evidence of extensive medial degeneration with elastin breaks, elastin lamellar thinning, smooth muscle cell dropout and diffuse glycosaminoglycan deposition. **E.** Mean diffusivity map from DTI scan corresponding to (D). **F.** Fractional anisotropy map from DTI scan corresponding to (D). DTI scan of aortic sample with limited medial degeneration displays relatively low mean diffusivity and relatively high fractional anisotropy. DTI parameter maps from aortic sample exhibiting extensive medial degeneration display a comparatively higher mean diffusivity and comparatively lower fractional anisotropy throughout the aortic media. The same colour scales are applied to each sample.
Figure 2.14 Relationship between DTI scalar metrics and elastin content in human ascending aortic tissue. A-C. Scatter plot with linear regression line depicting relationship between mean diffusivity (A), mean diffusivity (B), radial diffusivity, (C) fractional anisotropy and histologically quantified elastin content. Regions of interest from individual patient aortas are denoted by the unique symbols. Histology from each patient was qualified from 10 equidistant regions of interest around the aortic ring.
averaged MD, RD and FA (Figure 2.14). Surprisingly, linear regression analysis did not identify a significant correlation between regional elastin content and mean diffusivity (P=0.36, \( R^2 = 0.009 \)). Similarly, regressing RD on elastin content also did not identify a significant correlation (P=0.15, \( R^2 = 0.025 \)). However, linear regression between FA and region-quantified elastin did reveal a positive correlation (P=0.001, \( R^2 = 0.118 \)).

2.3.8 Diffusivity is inversely correlated to smooth muscle cell density

I next evaluated the relationship between DTI scalar indices and medial smooth muscle cell density (Figure 2.15). Linear regression analyses indicated that both MD and RD were inversely correlated with regional cell density (P<0.0001, \( R^2 = 0.18 \), \( \beta = -0.65 \); P=0.0005, \( R^2 = 0.14 \), \( \beta = -0.53 \)). No significant correlation between histologically-quantified cell density and fractional anisotropy was identified (P=0.22, \( R^2 = 0.02 \)).

2.3.9 Glycosaminoglycan content is assessed well by DTI scalar maps

Ascending aortic aneurysms are associated with local and variable extents of glycosaminoglycan deposits in media, which was also evident in our human cohort. This allowed for an additional histological metric of degeneration to be assessed, a pathology that was not be modelled by our porcine model, due to the ex vivo nature of the intervention. A regional assessment, as was performed in Figure 2.14 and 2.15, was employed to compare histological GAG content to voxel-averaged DTI scalar metrics. Figure 2.16A shows the correlation between region-quantified GAG and the
Figure 2.15 Relationship between DTI scalar metrics and smooth muscle cell density in human ascending aortic tissue. A-C. Scatter plot with linear regression depicting relationship between mean diffusivity (A), mean diffusivity (B), radial diffusivity, (C) fractional anisotropy and histologically quantified smooth muscle cell density. Regions of interest from individual patient aortas are denoted by the unique symbols. Histology from each patient was annotated with 10 equidistant regions of interest around the aortic ring.
corresponding voxel-averaged mean diffusivity. We noted a positive correlation (P<0.0001, $R^2=0.30$, $\beta=0.0007$). Figure 2.16B shows a similar strong positive correlation between region-quantified GAG and corresponding voxel-averaged radial diffusivity (P<0.0001, $R^2=0.34$, $\beta=0.0010$). Furthermore, I found an inverse correlation between ROI GAG content and voxel-averaged FA (P<0.0001, $R^2=0.19$) (Figure 2.16C).

2.3.10 2D tensor angles do not indicate the extent of medial degeneration in human aortic samples

I then sought to determine if medial degeneration can be correlated with 2D tensor angles as was the case in the porcine model. To do so, I chose aortas of patients 370 and 361, which showed very different compositions in their aortic media. Full-thickness, Movat’s pentachrome-stained aortas from patient 370 and 361, (Figure 3.17 A&B respectively) show different medial architectures. Aorta 370 had strong smooth muscle stain, a high density of intact elastin layers and scattered small GAG deposits; while aorta 361, displayed extensive elastin breaks, loss of smooth muscle and the presence of widespread glycosaminoglycan accumulation. However, in spite of these stark differences in medial architecture, 1$^o$ eigenvectors in both maps appear to conform to the circumferential axis with 2D tensor angles remaining close to 0$^o$ (Figure 2.17 C,D). Further quantitative regional analyses, following the same methods as in Figures 2.13-2.15, revealed no evidence for a statistically significant association between the average of absolute voxel 2D tensor angles and elastin content and voxel-averaged 2D tensor
Figure 2.16 Relationship between DTI scalar metrics and glycosaminoglycan (GAG) content in human ascending aortic tissue. A-C. Scatter plot with linear regression depicting relationship between mean diffusivity (A), mean diffusivity (B), radial diffusivity, (C) fractional anisotropy and histologically glycosaminoglycan content. Regions of interest from individual patient aortas are denoted by the unique symbols. Histology from each patient was evaluated in 10 equidistant regions of interest around the aortic ring.
Figure 2.17 Ascending aorta histology and DTI-derived 2D tensor angle maps.
A. Movat’s pentachrome-stained histology from human ascending aorta showing little evidence of medial degeneration with SMCs and thick elastin fibers. B. Movat’s pentachrome-stained histology from a sample showing irregular morphology, extensive medial degeneration with frequent elastin breaks, elastin lamellar thinning, SMC dropout as well as subintimal and diffuse GAG deposition C. Single slice 2D tensor angle map from DTI scan corresponding to (A). D. Single slice 2D tensor angle map from DTI scan corresponding to (B). The tensor angle maps do not reflect differences identified by photomicrographs of medial histology.
Figure 2.18 Relationship between absolute 2D tensor angle and measures of histological degeneration in human ascending aortic tissue. A. Scatter plot and linear regression depicting the relationship between ROI absolute 2D tensor angle and histologically quantified elastin content. B. Scatter plot and linear regression depicting the relationship between ROI absolute 2D tensor angle and histologically quantified cell density. C. Scatter plot and linear regression depicting the relationship between ROI absolute 2D tensor angle and histologically-quantified glycosaminoglycan (GAG) content. Region-averaged absolute 2D tensor angle indicates the extent of deviation of tensor orientations from aortic morphology. Lack of significant correlation indicates that tensor angles do not predict the extent of any markers of histological degeneration.
angles (Figure 2.18A, P=0.63, R²=0.003), cell density (Figure 2.18B, P=0.78, R²=0.001),
or regressions GAG content (Figure 2.18C, P=0.30, R²=0.013).

2.3.11 Regional variations in diffusivity at 9.4 T are reproduced at 3 T

To begin to evaluate the feasibility of in vivo imaging of human aorta, I
performed a DTI scan on sample 380 at a field strength of 3 T. This scan was performed
within two hours of the 9.4T scans. Figure 2.20 A, B depict a single slice from a DTI
scan, and the corresponding MD scalar map, performed at a field strength of 3T. Figure
2.20 C,D depict, from the same aorta, a single slice from a DTI scan, and the
corresponding MD scalar map, performed at a field strength of 9.4 T. Both The 3T and
9.4T MD scalar maps revealed a stronger signal intensity in the right half of aorta than
that in the left, as oriented in Figure 2.19. When co-aligned, the MD scalar map from the
3T scan also showed a similar pattern in MD magnitudes. As such we chose ROIs of
relatively low and high mean diffusivity to compare between 9.4T and 3T scans
(annotated as red and green regions in Figure 2.19 A,C). I compared the voxel-averaged
mean diffusivity in the matched regions (Figure 2.20E). From the 9.4T MD scalar map
there was a 34% reduction in MD between the two ROIs (P<0.0001). From 3T scalar
map there was a 44% reduction in voxel-averaged MD between the two ROIs
(P<0.0001).
Figure 2.19 Mean diffusivity scalar maps generated from scans at 3 T and 9.4 T field strengths. A. A slice from a DTI scan of human aneurysmal ascending aortic sample performed at a field strength of 3 T. B. Mean diffusivity scalar map derived from (A). C. A slice from DTI scan of the identical sample as in (A,B) performed at a field strength of 9.4 T. D. Mean diffusivity scalar map derived from (C). E. Bar graph comparing mean diffusivity in ROI 1 and ROI 2 (red and green squares respectively on DTI scans) in 9.4 T and 3 T scans.
2.4 Discussion

These findings demonstrate that DTI can be used to interrogate the microarchitecture of human ascending aortic tissue at a field strength of 9.4 T. In a porcine model with localized aortic medial damage, DTI scalars and voxel diffusion orientations correlated with histologically-defined aortic degeneration. In human ascending aortas with varied medial degenerative pathologies, DTI scalars correlated with a subset of histological readouts of aortic medial degeneration, most consistently with GAG content.

2.4.1 DTI characterization of the healthy porcine aorta

I first established that aortic tissue has diffusion-restricting attributes such that it can be studied by DTI. This supports the existing small body of research on DTI of the vasculature.\textsuperscript{18,24,25} It also consolidates the premise that not only is the heart a structure that can be evaluated by DTI\textsuperscript{26–28} but the much thinner aortic wall holds promise as well. A porcine model was chosen recognizing that aortic lamellar units are conserved within mammalian species, with the total number of lamellar units correlating to aortic size.\textsuperscript{29} As well the porcine aortic size and elastic properties are similar to humans.\textsuperscript{30–32}

Diffusion measurements of healthy pig aortas yielded mean MD, RD and FA values of $0.00065 \pm 0.00024 \text{mm}^2/\text{s}$, $0.00056 \pm 0.00027 \text{mm}^2/\text{s}$, and $0.43 \pm 0.15$ respectively. These values indicate ordered tissue that restricts free diffusion. As a comparison, the previous study on human myocardial tissue reported MD and FA of $0.00085 \text{mm}^2/\text{s}$ and 0.54, respectively.\textsuperscript{33} Moreover, the homogeneous medial appearance
of DTI scalar maps, which contrasts to the adventitia, is a further indication of the
detection of the dense ordered tissue present in the aortic media.

I also analyzed the orientation of diffusion tensors in healthy porcine aortas. The
1° eigenvector map shows a circumferential alignment of tensors around the aortic ring.
This pattern corresponds to the circumferential orientations of aortic structural
components-smooth muscle cells and elastin. I quantified the voxel-based tensor
orientation as the angular deviation from aortic tangent, termed 2D tensor angle. 2D
tensor angle in control porcine aorta was normally distributed, with a peak occurring at
0.32°, indicating a tight conformity of diffusion tensor orientation to the circumferential
morphology of the aortic ring. Moreover, 3D fiber tractography confirmed the continuous
nature and alignment of diffusion tensors throughout the aorta. The modeled fibers
spanned 51% of the aortic circumference, indicating a strong continuity in diffusion
orientations. Interestingly, the modeled exterior fibers (Figure 2.5C) displayed a slight
pitch to fiber orientations. This observation in fact corroborates the findings that aortic
vascular smooth muscle cells wrap helically. During the development of 2D tensor
angles, we considered the assessment of helix pitch angles. This would be informative
regarding changes in orientation and extent of diffusion along the length of the vessel in
the absence of smooth muscle cells and restrictive cell membranes. However, there is no
current consensus on the exact pitch angle in ascending aortic smooth muscle cell
alignment, as such it would not be possible to assess deviation in the z-axis from a known
reference pitch angle. This use of principal eigenvector orientations relative to a known
physiological marker, was ultimately derived from the use of helix angles in myocardial
DTI studies.\textsuperscript{39,40} In these cases, there were detectable changes in the prominent 1° eigenvector orientations in response to tissue damage, a phenomenon which we ultimately hoped could be replicated in our porcine model and diseased samples.

\textbf{2.4.2 DTI detects and quantifies damage in a porcine enzyme-induced model of medial degeneration}

I found that diffusion scalar maps demonstrated stark changes in DTI scalars at sites of injection. Additionally, these changes in DTI scalars seemed to outline the approximate morphology of lesion as visible in Movat’s and H&E-stained histology. We found that as elastin was lost, there was a significant, linear increase in mean and radial diffusivity and a significant linear decrease in fractional anisotropy. In a similar manner, quantified loss of smooth muscle cell density resulted in a significant, linear increase in mean and radial diffusivities and significant linear decrease in fractional anisotropy. These results corroborate and extend the findings of Tornfoglio et al., who found broad increases in MD and decreases in FA in a carotid artery model of arterial architecture change.\textsuperscript{17} These changes were in response to unquantified reductions in elastin and smooth muscle cell density, and thus lacking any linear correlations as I have performed in the present study. Within my porcine injection study, the regression coefficient was larger in absolute magnitude for RD than MD regressions in the histological analysis of both SMC density and elastin content. This indicates a larger increase in RD than MD for a given loss of elastin or cell death. Conceptually, we would expect increased interlamellar spacing in these cases. The loss of cell membranes would allow increased
diffusion of water along and perpendicular to 1° eigenvector. Increases in RD likely occurred due to the absence of restrictive cell membranes, allowing increased diffusion, not only in the interlamellar space as depicted by cross-sectional Movat’s pentachrome-stained histology, but also along the length of the vessel, approaching successive smooth muscle cell cross-sectional layers. This multifaceted change may account for the disproportionate increase in radial diffusivity compared to mean diffusivity and also, conceptually axial diffusivity.

Visually, enzyme-induced medial degeneration was detectable on 1° eigenvector maps. This was further illustrated by deviation from the normal, approximately 0° 2D tensor angle throughout the aortic ring. Quantification of all aortic injection samples resulted in a larger standard deviation within the distribution of 2D tensor angles in porcine-injected samples than healthy porcine aortas. There was also an increased incidence of tensor angles at higher absolute angles (above 40°) in injected porcine aortas. This increase at higher angles was outside of the modeled Gaussian distribution and largely due to the disproportionate change in 1° orientations at the site of injection.

**2.4.3 DTI detects features of medial degeneration within human aortopathy samples**

My studies are the first to explore whether DTI can provide valid insights into human TAA pathology. The sample cohort consisted of 8 human ascending aortas, which displayed a range of medial pathologies.
Indeed, heterogeneity in DTI scalars among different individual aorta was present. Heterogeneity in DTI scalars among the different regions of a given aortic ring was also seen. To understand what each DTI scalar, MD, RD, FA, measures in terms of aortic medial pathological features, I evaluated the relationship between voxel-averaged DTI scalars and quantified histological endpoints (elastin, smooth muscle cell and GAG) using a co-registration regional analysis framework across the whole aortic circumference.

Surprisingly, and in contrast to the porcine aortic data, elastin content did not significantly correlate with any DTI scalar metrics (MD, RD, FA). This discrepancy with the porcine model may be attributed to several reasons: 1) With the porcine model, there was a wider range of elastin content than in human diseased samples. That is, within the porcine injection model, elastin content ranged from entirely normal to the obliteration of essentially all cell and elastin components. Within our human cohort, while there was a wide range of elastin degeneration, elastin fidelity did not approach the extent of degeneration at the mid-injection zone within our model. 2) For the porcine model, elastin loss closely accompanied cell loss. While it has been well documented that these two phenomena can often present together in instances of ascending aortic aortopathy\textsuperscript{9,14}, there can also be regions of elastin degradation that are hypercellular. 3) While elastin is certainly a marker of medial degeneration and will accompany other histopathological aspects of medial degeneration, elastin itself is not likely a direct determinant of change in diffusion characteristics within the diseased aortic media. Elastin is not inherently a water-containing entity, rather a planar structure generated by the complex tertiary
interaction of multiple tropoelastin proteins. This elastin layering might act as a barrier to diffusion. However, given the presence of water restricting, inter-lamellar smooth muscle cells, the presence of elastin may have minimal effect on the bulk water diffusion within the aortic media.

Smooth muscle cell content inversely correlated with voxel-averaged MD and RD, but not FA. This is different from the significant correlation between FA and cell density identified in the porcine aorta model. However, there was a key human TAA histopathological phenomenon which was not well captured by our porcine model. Specifically, while the porcine medial degeneration model was effective at inducing cell loss, there were few regions encompassing smooth muscle cell disarray. Conversely, in our human cohort there were many regions of relative hypercellularity with smooth muscle cell disarray. Given the premise that diffusion, especially in cardiac tissue, is restricted by bipolar, elongated smooth muscle cell orientation and membranes, the presence of smooth muscle cell disarray may affect the quantification of fractional anisotropy. When aligned, as in healthy aortic tissue, we anticipate a disproportionate degree of diffusion along the axis of the aligned aortic components. However, in the presence of smooth muscle cell disarray, these water-containing, anisotropic membranes now are no longer aligned with the 1º eigenvector in a voxel and thus may contribute to a relative reduction in FA when compared to an equivalent case of aligned smooth muscle cells. This phenomenon can be paralleled to the better performance of diffusivity-based DTI scalar metrics in the detection of breast cancer tumours and lesions in comparison to fractional anisotropy. It was found that regional hemorrhage or loose connective tissue
present in tumours can be detected via mean diffusivity. However, FA detection of
tumours was dependant on loss of cellular organization due to the extent of liquefactive
necrosis, which varies largely depending on the nature and aggressiveness of the
tumour. As in the case of this study, varied presentations of cellular organization
phenotypes within a disease affected fractional anisotropy assessments, but not mean
diffusivity assessments.

GAG content correlated with DTI scalars. MD and RD positively correlated with
GAG content. FA inversely correlated to GAG content. We recently have established that
medial GAG content serves as a biomarker of aortic medial degeneration in human
ascending aortic aneurysm. Excess GAG deposition has several detrimental impacts to
aortic media integrity. GAG pooling correlates with smooth muscle cells loss at the site
of deposition. The presence of non-native glycosaminoglycan has also been suggested to
mediate smooth muscle cell death. Additionally, the structure of GAG produces highly
hydrated aggregates occupying a large volume of extracellular space. The increase in
water diffusivity with increased GAG content can be explained by the environment
generated by the presence of glycosaminoglycan. GAGs result in large, hydrated, non-
dense regions lacking any membranes to restrict diffusion. This environment may be
conducive to increases in axial diffusivity, and possibly more so, radial diffusivity. This
phenomenon is supported by 37% difference in regression coefficient when comparing
RD and MD (β=0.0010 and 0.0007 respectively). This larger change in RD, likely
occurred due to the absence of restrictive cell membranes in areas with high GAG
content. Increased diffusion can be expected, not only in the interlamellar space as
depicted by cross-sectional Movat’s pentachrome-stained histology, but also along the length of the vessel, approaching successive smooth muscle cell cross-sectional layers. This multifaceted change in may account for the disproportionate increase in radial diffusivity compared to mean diffusivity and also, conceptually axial diffusivity. The reduction of FA in the presence of GAG may be attributed to a reduced directional constriction of diffusion in the absence of SMCs.

2.4.4 2D tensor angles do not detect the extent of medial degeneration in human samples

Although 2D tensor angles reflected damage in the porcine model, they did not readily identify human aortic medial degeneration. In two aortas with different smooth muscle cell density, elastin content and glycosaminoglycan content, the distribution of 2D tensor orientations was similar and close to 0°. In the co-registered regional assessments, the absolute magnitude of a given 2D tensor angle (as an indication of deviation from the expected normal tensor angle of 0°) did not correlate with elastin content, smooth muscle cell density, or glycosaminoglycan density. Thus, the predominant 2D orientation of diffusion tensors do not vary significantly with medial degeneration within the aortic media. It may be that only the pathology of highly diseased specimens could change the medial composition sufficiently as to alter predominant 2D diffusion tensor orientations. The presence of independent structures (elastin, collagen, smooth muscle cells) each broadly oriented circumferentially may limit net changes in 2D diffusion angles. This result is quite different from what has been observed in the myocardium, which has
informed myocardial helix angles. Myocardial helix angles have been correlated with the presence of myocardial infarction or restructuring.\textsuperscript{45–47}

2.4.5 DTI performed at 9.4 T and 3 T yield similar estimations of mean diffusivity

As a first step in considering the translational potential of aortic DTI, we assessed the same human aortopathy sample at a field strength of 9.4 T and 3 T. We achieved a spatial resolution of 800 x 800 µm, equivalent to 2-3 voxels per full-thickness aortic wall. A direct comparison between quantified DTI scalars at 3 T and 9.4 T is difficult due to their different b-values (b=350 s/mm\textsuperscript{2} and 1000 s/mm\textsuperscript{2} for 3T and 9.4T scans respectively) and the impacts of b-values on DTI scalar calculation.\textsuperscript{48} However, we observed a similar pattern of spatial heterogeneity of mean diffusivity maps derived from both scans. Moreover, co-alignment comparison of regional mean diffusivity was similar. This offers some confidence that DTI performed at 3 T merits further study.

2.5 Limitations

There are many factors to consider if aortic DTI is to be realized clinically. For this proof-of-principle study we primarily imaged human ascending aortas \textit{ex vivo} and with high magnetic field strength MRI. This approach yielded excellent spatial resolution. However, this resolution is not currently achievable on 1.5T and 3T MRI scanners. Tissue thickness is also relevant. DTI has been performed \textit{in vivo} to assess the myocardium, which has a thickness of 7-14 mm in healthy adults.\textsuperscript{49} The ascending aortic wall is approximately 2 mm in thickness.\textsuperscript{50} To date cardiac DTI \textit{in vivo} has achieved a
spatial resolution of 2.2-2.8 mm$^2$ on 3T scanners.$^{26,33,51}$ This may be insufficient to assess the aortic wall. Our study was able to achieve an 800 x 800 μm spatial resolution through the use of a wrist radio frequency coil. Achieving a similar resolution in the body when using a body coil with a phased array detector is an area of future development. In addition, *in vivo* aortic DTI assessments would require precise cardiac and respiratory gating to limit motion artifacts.

DTI scans of human aortas were performed on formalin-fixed tissue in an effort to maintain tissue integrity for post-scan histological analysis. Because of this, direct comparison of absolute DTI scalar metric magnitudes to porcine aortic tissue is not feasible. Limited studies have assessed the effect of formalin fixation on DTI scalar metrics. Giannakidis et al. have previously noted that fixation less than 2 weeks of rat cardiac tissue offered highly consistent readouts of DTI scalar metrics, despite changes in values compared to fresh tissue.$^{52}$ This result is consistent with our experimentation of the effect of fixation on DTI scalars metrics in porcine aortic tissue.

**2.6 Summary**

I have developed high-field-strength assessments of the microarchitecture of ascending aortic tissue samples via DTI. We quantitatively established that DTI scalars are capable of detecting and quantifying elements of aortic medial degeneration in a porcine model and in human ascending aortas. Furthermore, we have discovered that DTI assessments are particularly well correlated with GAG accumulation in aortic media. These findings offer a potentially novel approach for assessing vulnerable ascending aortic disease.
2.7 References


CHAPTER 3

3.1 Overview

In this thesis I examined the relationships between quantifiable diffusion tensor imaging metrics and elements of aortic medial degeneration. A DTI protocol was optimized and applied to healthy porcine aortas, locally damaged porcine aortas, and finally a cohort of human aortic aneurysm specimens. Mean diffusivity, radial diffusivity and fractional anisotropy were demonstrated to be sensitive to certain aspects of medial degeneration while, inconsistencies between correlations in our porcine medial degeneration model and our human cohort were logically explored. This work may serve as a potential pilot study for the use of DTI scalars as potential biomarker to the extent of thoracic aortic disease and the risk of aortic complications.

3.2 Summary and Conclusions

In this thesis I tackled a critical problem in understanding TAA pathophysiology. DTI as a potential imaging modality of the aorta has provided a potential avenue for improved TAA risk assessment beyond lumen dimensions. This is critical as no other imaging modality to-date has succeeded in providing insights into the extent of medial degeneration within the aortic wall, a key underlying factor in aortic catastrophes.

I have established the structure of the ascending aorta of adult pigs and humans is such that can be productively interrogated by DTI. Specifically, I have made 5 major findings:
1. The healthy porcine aorta has diffusion attributes that are indicative of a tissue that is restrictive to free diffusion. I established the DTI scalar magnitudes of the normal porcine ascending aorta, specifically for FA, MD and RD at a b-value of 1000 s/mm\(^2\).

2. The orientation of diffusion within the normal porcine ascending aorta is largely circumferential. Diffusion tensors are tightly aligned such that 3D fiber tractography yield circumferentially oriented tracts throughout the aortic media. The slight pitch of fiber tracts lends support to the helical wrapping of smooth muscle cells in the aortic wall.

3. I discovered that DTI at 9.4 T is a powerful tool for detecting a wide range of architectural disorder in the porcine aorta. DTI was found to quantitatively distinguish differing levels of degeneration.

    This finding supports a very recent study on the porcine carotid artery\(^2\) and uniquely provides quantitative data on the correlation of DTI metrics to pathologic attributes.

4. I discovered that human ascending aortic aneurysms can be imaged \textit{ex vivo} using DTI. I further identified wide inter-individual variability in quantitative DTI indices.

5. I discovered that quantitative DTI indices particularly correlated with the content of aortic wall components. Specifically, I found that the abundance of GAGs correlated with DTI scalar metrics. GAG content was inversely correlated with FA and positively correlated with MD and RD.
We concluded that DTI assessments are sensitive to elements of thoracic aortic disease, with DTI scalars being particularly sensitive to the presence of GAGs in diseased human ascending aortic tissue.

3.3 Limitations

I utilized ultra-high-field MRI to perform high resolution DTI assessments of *ex vivo* aortic samples. However, when considering translation to *in vivo* studies DTI resolution at 3T must be considered. Significant advances in DTI imaging at 3T would be required in order to reliably image the ascending aorta *in vivo*. DTI scalar sensitivity to changes in cellular density and glycosaminoglycan content were detected through comparison of regions with a multitude of voxels. However, realistically, given currently achievable thoracic DTI resolution, the aortic wall thickness would only contain 1-2 tissue containing voxels. Assessments of aortic DTI *in vivo* would demand a higher resolution that has been shown in existing thoracic DTI studies in order to reliably characterize the aortic wall.

Within the current study, a limited human cohort of 8 ascending aortic samples was studied. While the aortas studied did encompass a wide variety of medial degeneration phenotypes, a larger cohort, potentially also expanding the study to include samples from genetic etiologies would add confidence to the trends noted.

Another important limitation to address is the histological validation of DTI scalar indices within this study. The relationships found between DTI scalar indices and histology were through an assessment of a 500 µm MRI slice and a 5 µm histological section. Myocytes will range from 17 to 25 µm in diameter. As such, roughly 25 smooth
muscle cell layers would be represented by DTI scalar intensities within the single slice analysis applied within this study. Furthermore, given variations in glycosaminoglycan deposition patterns (i.e. diffuse, or pooling), there are likely cases where the measured histological GAG content of an ROI is not representative of the GAG content for the equivalent MRI slice thickness. Extensive efforts were taken to ensure that the appropriate slice was selected to correspond to the histological section used for comparison. Despite this, the disparity in the thickness of histological sections and MRI slices is a potential limitation for the accuracy of comparisons performed within this study.

Within this study, the quantification of both elastin content and glycosaminoglycan content was derived from colour threshold assessment of Movat’s Pentachrome stain from digital histological sections. Here, an identical staining protocol was used for all samples. With this being said however, the potential exists for staining variation between samples and the masking of certain histological components. The use of a pentachrome stain allows for the co-assessment of multiple aortic components from a single section. While this permits visualization of the complex nature of ascending aortic medial organization, it may also lead to mis-quantification of individual aortic components. For example, the dominant, black staining of elastin could overshadow the staining of GAGs in Movat’s pentachrome. An alternative approach to verify the trends found by our regional correlations would be an assessment using component-specific stains such as alcian blue staining for glycosaminoglycan or a Verhoeff’s elastin stain.
3.4 Future directions

The findings in Chapter 2 have indicated that DTI scalars can quantifiably assess the presence of glycosaminoglycan, and in the case of diffusivity-based scalars also predict the likely cellularity of a given aortic tissue. We suggested, in the assessment of glycosaminoglycan by MD and RD, that a large portion of changes in diffusivity occurred due to a disproportionate change in radial diffusion. In the future it would be interesting to understand the extent to which diffusion in the direction of the vessel contributes to this change in radial diffusivity. This approach however would require precise control of the orientation of slicing of aortic samples to ensure the excision of aortic rings occurs in a perpendicular orientation to the aortic lumen.

Another potential future direction of this work would be the further investigation in the assessment of aortic DTI in vivo at a field strength of 3 T. I would suggest the continued joint assessment of human aortic samples to confirm the reliability of 3 T assessments within the optimized scan parameters. Once confirmed, in vivo optimization and trials could begin with a focus first on cardiac and respiratory gating and subsequently achieving a reasonable resolution to assess the aortic wall, while maintaining a strong diffusion weighting.

3.5 Significance and Impact

It is important to recognize the challenges present in the existing framework for TAA assessment. A lack of any biomarker for aortic wall integrity leaves TAA patients at risk of adverse aortic complications. This work contributes significantly to the literature as it is a novel study, which demonstrates the sensitivity of DTI scalars to
histopathological markers of disease within the aortic wall. We are the first to take a ground truth approach using quantified, histology-based metrics of medial degeneration to verify the sensitivity of DTI scalars to changes in aortic wall architecture. To our knowledge, this is the first DTI study of human aortic samples within the framework of thoracic aortic disease. This work has significant impact as it can potentially lead to a non-invasive imaging framework to assess the risk of aortic complication beyond lumen dimensions.
3.6 References


4 APPENDICES

4.1 Appendix A: Health Science Research Ethics Board Approval Notice

Date: 23 July 2021
To: Geoffrey Pickering
Project ID: 5512
Study Title: Identifying the relationship between molecular determinants and age-related disorders in the human population 15405E
Application Type: Continuing Ethics Review (CER) Form
Review Type: Delegated
REB Meeting Date: 08/Aug/2021
Date Approval Issued: 23/Jul/2021
REB Approval Expiry Date: 13/Aug/2022

Dear Geoffrey Pickering,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB00006940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

The Office of Human Research Ethics

*Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).*
4.2 Appendix B: Curriculum Vitae

JUSTIN CHING-JOHNSON

Education

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<tr>
<th>Year</th>
<th>Degree</th>
<th>Institution</th>
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<tr>
<td>2018-present</td>
<td>Master of Science in Medical Biophysics</td>
<td>University of Western Ontario</td>
</tr>
<tr>
<td>2014-2018</td>
<td>Honours Bachelor in Medical Sciences (Specialization in Biochemistry)</td>
<td>University of Western Ontario</td>
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Professional/Work Experience

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<tr>
<td>2018-Present</td>
<td>Graduate Student</td>
<td>Pickering Lab, Dept. of Medical Biophysics, University of Western Ontario</td>
<td>- Initialized and optimized an MRI approach to assess compartmentalized assessment of aortic wall integrity</td>
</tr>
<tr>
<td>2018-2019</td>
<td>Teaching Assistant-Biology 1001A</td>
<td>Department of Biology, University of Western Ontario</td>
<td>- Conducted intro biology tutorials. - Taught introductory genetics, biochemistry and ecology concepts.</td>
</tr>
<tr>
<td>2016-2018</td>
<td>Undergraduate Researcher</td>
<td>Junop Lab, Dept. of Biochemistry, University of Western Ontario</td>
<td>- Elucidation of the nuclease activities of protein SNM1A</td>
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Research Summary

<table>
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<th>Research Summary</th>
<th>Authors</th>
<th>Institution</th>
<th>Description</th>
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<tr>
<td>2018-Present</td>
<td>Assessment of thoracic aortic structure and integrity via novel MRI modalities</td>
<td>Dr. Geoffrey Pickering, Dr. Robert Bartha, Robarts Research Institute, University of Western Ontario</td>
<td>Sought to assess the ability of various unique MRI modalities (Diffusion Tensor Imaging, Chemical Exchange Saturation Transfer) to assess the degree of disease in the aortic wall</td>
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2016-2018 Assessment of SNM1A mechanistic activity and inhibition in inter-strand crosslink repair and chemotherapeutic resistance
Dr. Murray Junop, Department of Biochemistry, University of Western Ontario
Sought to determine the specific mode of action of the exo/endo-nuclease SNM1A in the framework of interstrand-crosslink repair and chemotherapeutic resistance

Presentations/Posters

2021 Robarts Research Retreat-Poster Presentation
Glycosaminoglycan imaging of ascending aortic aneurysms with chemical exchange saturation transfer MRI

2020 GenTAC Aortic Summit-Oral Presenter
Glycosaminoglycan imaging of ascending aortic aneurysms with chemical exchange saturation transfer MRI

2020 Schulich Medicine Resident Research Day-Poster Presentation
Assessment of aortic degeneration by diffusion tensor imaging

2019 London Health Research Day- Poster presentation
Assessment of aortic wall architecture by novel magnetic resonance imaging strategies

2019 Robarts Research Retreat-Poster Presentation
Assessment of aortic degeneration by diffusion tensor imaging

Awards

2018 Western Graduate Research Scholarship (WGRS)
Graduate GPA-based scholarship
Value: $5000

2017 Dean’s Undergraduate Research Scholarship
Summer research stipend award given to undergraduate students across various disciplines
Value: $3000

2014 Western Scholarship of Distinction
Undergraduate entrance scholarship
Value: $3500
Accepted  Rokhsana Mortuza*, Justin Ching-Johnson*, Hao Yin*, Caroline O’Neil, Alicia E. Cronin, Varinder Randhawa, Zengxuan Nong, Abdulaziz Ahmed Hashi, Alex X. Li, Robert Bartha, Michael W. A. Chu, J. Geoffrey Pickering. Imaging of glycosaminoglycans in ascending aortic aneurysms with chemical exchange saturation transfer MRI JACC Cardiovascular Imaging